UDK 63/66 (058)0808.1/2 BH ISSN 0033-8583

RADOVI

POLJOPRIVREDNO-PREHRAMBENOG FAKULTETA UNIVERZITETA U SARAJEVU

WORKS

OF THE FACULTY OF AGRICULTURE AND FOOD SCIENCES UNIVERSITY OF SARAJEVO

Godina / Volume LXII

Broj / No. 67/2

Sarajevo, 2017.

Uredništvo i administracija - *Address of the Editorial Board:*
Poljoprivredno-prehrambeni fakultet Univerziteta u Sarajevu / *Faculty of Agriculture and Food Sciences University of Sarajevo*
Zmaja od Bosne 8
71000 Sarajevo
Bosna i Hercegovina

Tel.:	+387 (0)33 225 727	
Fax.:	667 429	
e-mail:	radovi@ppf.unsa.ba	
Transakcijski račun br:	3389002208130126	UNICREDIT BANK
IBAN:	BA393389104801155558	UNICREDIT BANK
SWIFT:	UNCRBA 22	

Radovi Poljoprivredno-prehrambenog fakulteta Univerziteta u Sarajevu
AGRIS - Agricultural Information Servis, 1959; CAB Publishing - UK, 2002.

Štampa:
„ŠTAMPARIJA FOJNICA“ d.o.o. Fojnica

Izdavač - *Publisher*:
Poljoprivredno-prehrambeni fakultet Univerziteta u Sarajevu / *Faculty of Agriculture and Food Sciences University of Sarajevo*

S A D R Ž A J / C O N T E N T

Stranica
Page

UTICAJ NORME SJETVE NA PRINOS ZRNA I SADRŽAJ UKUPNIH FENOLA U OBIČNOJ HELJDI*

EFFECTS OF SOWING RATE ON YIELD AND TOTAL PHENOLIC CONTENTS OF COMMON BUCKWHEAT

Teofil Gavrić[1], Drena Gadžo[1], Mirha Đikić[1], Zilha Ašimović[1]

Original scientific paper

Rezime

Obična heljda (*Fagopyrum esculentum* Moench) je jednogodišnja vrsta iz familije *Polygonaceae*. Norma sjetve heljde po jedinici površine se kreće od 40 do 100 kg ha^{-1}, te može imati značajan uticaj na prinos zrna i sadržaj ukupnih fenola. Cilj ovog istrtaživanja je bio utvrditi kako različite norme sjetve utiču na prinos zrna i sadržaj ukupnih fenola u heljdi. U trogodišnjem istraživanju (2011-2013) ispitivane su tri različite norme sjetve (50, 80 i 100 kg ha^{-1}). Poljski ogled je bio postavljen u Donjem Selu, i to po principu slučajnog blok sistema sa četiri ponavljanja (Općina Ilijaš). Utvrđeno je da istraživane norme sjetve nisu značajno uticale na visinu biljaka, ali su imale negativan uticaj na apsolutnu masu. Vrijednost hektolitarske i apsolutne masa su varirale u zavisnosti od godine istraživanja. Prinos zrna je značajno zavisio od godine istraživanja i norme sjetve. Najniži prinos zrna je zabilježen u varijanti sa najmanjom normom sjetve (812,0 kg ha^{-1}), a najveći u varijanti sa najvećom normom sjetve (1428,9 kg ha^{-1}). Sadržaj ukupnih fenola je bio u granicama od 0,34 do 4,19 mg g^{-1} i zavisio je od godine istraživanja.

Ključne riječi: *obična heljda, norma sjetve, prinos, ukupni fenoli*

Summary

Common buckwheat (*Fagopyrum esculentum* Moench) is an annual plant from *Polygonaceae* family. Sowing rate ranges from 40 to 100 kg of seed per hectare and it can have a significant effect on the yield of grain and total phenolic contents. The main focus of this research was to determine how change of sowing rate reflects on the yield and total phenolic contents of buckwheat. During three years (2011-2013) three different rates of seed were used: 50, 80 and 100 kg ha^{-1}. Buckwheat was sown in the village Donje Selo, near Ilijaš and the experiment was set up in four repetitions. The sowing rate did not have a significant effect on plant height, but it had a negative effect on the mass of 1000 kernels. Hectoliter mass and the mass of 1000 kernels

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Poljoprivredno-prehrambeni fakultet, Univerziteta u Sarajevu, t.gavric@ppf.unsa.ba

varied in dependence on the years of investigation. Grain yields were significantly dependent on the year of investigation and sowing rates. The lowest grain yield was recorded in the variant with the lowest sowing rate (812.0 kg ha^{-1}), and the largest grain yield was recorded in variant with the highest sowing rate (1428.9 kg ha^{-1}). Phenol contents in kernels ranged from 0.34 to 4.19 mg g^{-1} and depend on years of investigation.

Key words: *common buckwheat, sowing rate, yield, total phenolic*

UVOD

Obična heljda (*Fagopyrum esculentum* Moench) je jednogodišnja vrsta iz familije *Polygonaceae.* Zrno obične heljde se primarno koristi za dobijanje brašna, prvenstveno zahvaljujući njegovom hemijskom sastavu, koji je sličan zrnu žita. Pored zrna koje se koristi za dobivanje brašna i niza proizvoda od njega, u novije vrijeme, zahvaljujući saznanjima o njenoj ljekovitoj funkciji, heljda dobiva sve veći značaj zbog upotrebe i ostalih dijelova biljke, prvenstveno lista i cvijeta. Posljednjih godina, kada se sve češće postavlja pitanje kakvu hranu koristimo u ishrani, heljda pronalazi svoje mjesto kao biljka za proizvodnju *funkcionalne hrane* tj. hrane koja ima izražena i ljekovita svojstva.

Heljda se uglavnom proizvodi u umjerenom klimatskom pojasu i brdsko-planinskim predjelima. Prema FAO podacima, u 2014. godini u svijetu se uzgajala na površini od oko 2 miliona ha. Do pedesetih godina prošlog vijeka uzgajala se i na području Bosne i Hercegovine, da bi potom u potpunosti isčezla iz proizvodnje. Razlog prestanka sjetve heljde poslije Drugog svjetskog rata je industrijalizacija zemlje i odlazak stanovništva iz ruralnih brdsko-planinskih područja u gradove, te uvođenje novog sortimenta žita (pšenice i kukuruza) koji su daleko viših i stabilnijih prinosa, pri čemu heljda gubi na značaju (Gadžo i sar., 2016). Međutim, nakon posljednjeg rata u Bosni i Hercegovini (1992-1995), zahvaljujući sve većem broju radova koji govore o ljekovitosti ove vrste, kao i njene sve veće upotrebe u farmaceutskoj industriji i medicini, ona se ponovo počinje proizvoditi na ovim prostorima. Danas se kod nas uglavnom uzgaja zbog zrna, ali sve više se proizvode i različiti heljdini preparati koji imaju ljekovitu funkciju. Ljekovitost heljde potiče od sadržaja fenolnih jedinjenja, odnosno rutina koji je najzastupljeniji u ukupnim fenolima. Sposobnost heljde da sintetiše fenolna jedinjenja je nastala kao evolucijski odgovor na nepovoljne uslove uspijevanja (Germ, 2004), a sadržaj fenola zavisi od vrste i sorte heljde (Bystrická i sar., 2010; Christa i Soral-Śmietana, 2008), nadmorske visine (Guo i sar., 2011), lokacije i uslova uzgoja (Sakač i sar. 2014; Suzuki i sar., 2005; Christa i Soral-Śmietana, 2008). Smatra se da konzumiranje heljde doprinosi ublažavanju ili sprečavanju mnogih bolesti današnjice. Korisna je njena upotreba u ishrani osoba oboljelih od dijabetesa (Wang i sar., 1992; Kawa i sar., 2003; Li i sar., 2016), pri povišenom krvnom pritisku (Choio i sar., 2001); djeluje kao antioksidans (Oomah i Maza, 1996); uspješno liječi kardiovaskularna oboljenja (Tomotake i sar., 2001a),

te posjeduje i antikancerogenu aktivnost (Tomotake i sar., 2001b; Park i Park, 2004).
Općenito, norma sjetve heljde po jedinici površine se kreće od 40 do 100 kg ha^{-1}, u zavisnosti da li se sije širokoredno ili uskoredno. Literaturni podaci su oprečni kada je u pitanju optimalan broj biljaka po jedinici površine (Gadžo i sar., 2007; Kwang i sar., 2007; Wang i sar., 1986; Kvashchuk, 1992; Sheng i Sheng, 1992). Norma sjetve može značajno mijenjati prinos zrna ali i uticati na sadržaj ukupnih fenola. U Bosni i Hercegovini je mali broj naučnih radova o ovom tipu istraživanja, te je na osnovu ove činjenice i postavljen cilj da se utvrdi kako norma sjetve utiče na agronomske osobine heljde i sadržaj ukupnih fenola u zrnu.

MATERIJAL I METOD RADA

U trogodišnjem periodu (2011-2013) ispitivana je obična heljda, sorta darja porijeklom iz Slovenije. Istraživanja su izvedena na lokalitetu Donje Selo, Općina Ilijaš (930 mnv), a laboratorijski dio istraživanja je urađen na Poljoprivredno-prehrambenom fakultetu Univerziteta u Sarajevu. U ogledu su ispitivane tri norme sjetve: 100 kg ha^{-1} (N1), 80 kg ha^{-1} (N2) i 50 kg ha^{-1} (N3). Poljski ogled je postavljen po slučajnom blok sistemu u četiri ponavljanja, a površina osnovne parcele je iznosila 4,8 m^2. Usjev je posijan kada se sjetveni sloj zemljišta zagrijao na oko 10°C. Sjetva je bila ručna na dubini 3-4 cm sa međurednim rastojanjem od 15 cm. U toku vegetacije korovi su uništavani ručno, a žetva je obavljena u fazi 2/3 zrelih plodova. Određivanje ukupnih fenola u heljdi određeno je spekrtofotometrijski modificiranom metodom po Folin-Ciocalteu (Bystrická i sar., 2010), za ekstrakciju je korišten 60% etanol, a standard je bila galna kiselina. Svi dobiveni podaci obrađeni su odgovarajućim matematsko-statističkim metodama uz korištenje SPSS 22 programa.

Vremenski uslovi

Na osnovu podataka prezentiranih u tabeli 1 vidljivo je da su srednje godišnje temperature u istraživanom periodu bile veće od višegodišnjeg prosjeka. U 2011. godini prosječna godišnja temperatura je bila viša za 1,0°C od višegodišnjeg prosjeka. Najtopliji mjesec u ovoj godini je bio avgust, što je za 2,7°C više od prosječne vrijednosti za ovaj period, dok su juni i juli imali srednju mjesečnu temperaturu višu za 1,5°C od višegodišnjeg prosjeka. U 2012. godini srednja godišnja temperatura je iznosila 8,1°C, što je za 1,7°C iznad višegodišnjeg prosjeka (6,4°C). Najtopliji mjesec u ovoj godini je bio juli (20,4°C) i u odnosu na višegodišnji prosjek bio je topliji za 4,4°C. Jun (18,7°C) je također imao za 4,4°C višu prosječnu temperaturu u odnosu na višegodišnji prosjek. U 2013. godini srednja godišnja temperatura je bila za 1,9°C viša u odnosu na višegodišnji prosjek. U ovoj godini najtopliji mjesec je bio avgust sa srednjom mjesečnom temperaturom koja je bila za 3°C viša u odnosu na prosjek. Povećanje temperatura u istraživanom periodu pratila je i suša tj. bilo je manje oborina, pogotovo u ljetnim mjesecima kad su usjevu bile najpotrebnije (tab. 1). U prvoj godini istraživanja (2011. godina) suma oborina je bila manja od višegodišnjeg prosjeka (805 mm) i iznosila je 626 mm. U druge dvije godine (2012. i 2013. godina) godišnja suma oborina je bila viša (861 i 905 mm), ali su oborine bile neravnomjerno raspoređene. Uglavnom, najviše oborina je palo u periodu bez vegetacije, dok je u toku vegetacije bila suša. Suša je posebno bila izražena tokom 2011. i 2012. godine u periodu cvjetanja i nalijevanja zrna kada je u avgustu zabilježeno 9,2 odnosno 0,6 mm oborina.

Tab. 1. Srednje mjesečne temperature i mjesečne sume oborina.
Average monthly temperatures and average amount of rainfall.

Godina	**Mjesec / Month**												**Prosjek Average**
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	
	Srednja mjesečna temperatura - Average monthly temperature (°C)												
1961-1990	-4,8	-2,2	1,6	6,4	11,4	14,3	16	15,5	12	7,2	2,1	-2,8	6,4
2011.	-2,5	-2,4	1,8	7,7	11	15,8	17,5	18,2	15,5	6,4	0,8	-1	7,4
2012.	-4,5	-7,2	3,6	7,4	10,9	18,7	20,4	19,7	15,1	9,5	6	-2,8	8,1
2013.	-0,7	0	2,3	8,9	12,5	15,6	18,1	18,5	12,3	9,4	4,7	-1,6	8,3
	Sume padavina - Amount of rainfall (mm)												**Suma Amount**
1961-1990	53	50	56	62	73	84	73	70	66	66	84	71	805
2011.	30,2	27,6	28,8	42,7	123	62,8	82,2	9,2	36,7	59,6	23,7	100,1	626
2012.	90,8	100,6	23,6	93,7	205,1	11,6	77,6	0,6	59,4	67	50,7	80,7	861
2013.	126,1	138,9	71,7	44	121,5	41,4	28,4	70,6	108	78,5	70,8	5,7	905

REZULTATI ISTRAŽIVANJA

Iz rezultata istraživanja (tab. 2) je vidljivo da je prosječna visina biljaka u trogodišnjem periodu (2011-2013) bila u rasponu od 101,3 do 114,1 cm. Statističkom analizom ustanovljeno je da norma sjetve nije značajno uticala na porast heljde, iako je bilo za očekivati da će ovaj faktor imati pozitivan uticaj. Značajan uticaj na ovu osobinu je imala godina istraživanja. U 2012. godini, odnosno u godini koja je početkom vegetacije imala visoku sumu oborina (maj 205,1 mm oborina) izmjerene su značajno više biljke (111,3 cm) u odnosu na druge dvije godine istraživanja. Sličan uticaj godine na porast biljaka je zabilježen i kod Glamočlije i sar. (2012). Autori su u relativno nepovoljnim vremenskim uslovima (nedostatak oborina) zabilježili za 8,2-13,0% niže biljke u odnosu na one uzgajane u povoljnim vremenskim uslovima. Uticaj godine istraživana, odnosno vremenskih uslova na visinu biljaka zabilježen je i kod Ikanović i sar. (2013) i Maletića i Jevđovića (2003).
Rezultati trogodišnjeg istraživanja koji su prezentirani u tabeli 2 pokazuju da je faktor godina istraživanja statistički značajno uticao na apsolutnu masu, te se ona kretala od 22,21 (2012) do 24,87 grama (2011). Niža vrijednost apsolutne mase u 2012. godini je najvjerovatnije bila posljedica smanjenog nalijevanja zrna izazvano sušnim periodom

u drugom dijelu vegetacije (tab. 1). Dobiveni rezultati se mogu potvrditi rezultatima istraživanja Alekseyeve i sar. (2001) koji su utvrdili da različiti agroekološki uslovi u pojedinim godinama imaju signifikantan uticaj na masu 1.000 zrna. Istraživani faktor norma sjetve u višegodišnjem periodu nije ispoljio statistički značajan uticaj na apsolutnu masu, ali je neznatno imao negativan uticaj na njenu vrijednost. To je u skladu sa istraživanjima Wanga i sar. (1986), a u suprotnosti sa rezultatima Shenga i Shenga (1992).

Tab. 2. Visina biljaka, apsolutna i hektolitarska masa.
Plant height, 1000-grain weight and hectoliter mass.

Norma sjetve	**Visina / Plant height (cm)**				**Apsolutna masa / 1000-grain weight (g)**				**Hektolitarska masa / Hectoliter mass (kg)**		
	2011.	2012.	2013.	Prosjek	2011.	2012.	2013.	Prosjek	2012.	2013.	Prosjek
N1	105,9	114,1	106,2	108,7^{ns}	24,7	22,8	22,5	23,3^{ns}	50,5	61,6	56,0^{ns}
N2	101,3	112,6	109,7	107,9^{ns}	25,1	22,1	22,6	23,2^{ns}	52,4	58,5	55,5^{ns}
N3	104,0	107,3	110,1	107,4^{ns}	24,9	21,7	22,5	23,1^{ns}	52,5	60,4	56,5^{ns}
Prosjek	103,7^{b}	111,3^{a}	108,6^{b}		24,87^{a}	22,21^{b}	22,53^{b}		51,80^{b}	60,15^{a}	

Hektolitarska masa koja je prvi pokazatelj kvaliteta zrna kretala se u rasponu od 50,5 do 61,6 kg. Nastale razlike u hektolirarskoj masi pod utjecajem norme sjetve su bile slučajne i nisu statistički opravdane, što je u skladu sa rezultatima istraživanja Varge i sar. (2000). Međutim, dobiveni rezultati se ne podudaraju sa rezultatima koje su dobili Bohle i sar. (1998). Ovi autori su ustanovili da gušća sjetva pozitivno utiče na vrijednost hektolitarske mase. Ukoliko se posmatra hektolitarska masa po godinama istraživanja, može se vidjeti da je u 2012. godini bila niža za 8,35 kg u odnosu na 2013. godinu i ta razlika je bila statistički značajna. Njena niža vrijednost u 2012. godini je bila najvjerovatnije posljedica nepovoljnih vremenskih uslova u drugom dijelu vegetacije koji su uzrokovali lošije nalijevanje, nejednaku veličinu i oblik zrna, a to su faktori koji direktno utiču na hektolitarsku masu.

Tab. 3. Prinos zrna i sadržaj fenola u zrnu
Kernel yield and phenol contents

Norma sjetve	**Prinos / Yield (kg ha^{-1})**				**Sadržaj fenola / Phenol contents (mg GAE g^{-1})**			
	2011.	2012.	2013.	Prosjek	2011.	2012.	2013.	*Prosjek*
N1	566,3	825,9	1.043,7	812,0^{a}	0,34	3,36	0,43	1,38^{ns}
N2	700,7	1.163,2	1.323,5	1.062,5^{b}	0,40	3,47	0,30	1,39^{ns}
N3	1.824,0	946,8	1.516,0	1.428,9^{c}	0,38	4,19	0,33	1,63^{ns}
Prosjek	1.030,3^{b}	978,7^{b}	1.292,7^{a}		0,37^{b}	3,67^{a}	0,35^{b}	

U trogodišnjem prosjeku prinos zrna je bio u značajnoj zavisnosti od gustine sjetve i godine istraživanja što se može vidjeti iz tabele 3. Prosječan prinos zrna u varijanti

ogleda N1 je bio 812,0 kg ha^{-1}, te je sa povećanjem norme sjetve porastao na 1062,5 (N2), odnosno na 1.428,9 kg ha^{-1} (N3). U relativnim pokazateljima povećanje prinosa je bilo za 23,6% (N2), odnosno 43,1% (N3). Sličan uticaj gustine sjetve zabilježen je i u pojedinim godinama. Ovim istraživanjem je ustanovljeno da povećanje norme sjetve uzrokuje povećanje prinosa što je u skladu sa istraživanjem koje su imali Sheng i Sheng (1992). Međutim, nekada povećanje norme sjetve može uzrokovati i opadanje prinosa uslijed polijeganja i propadanja usjeva (Gadžo i sar., 2007; Vilcāns i sar., 2013). Značajna variranja prinosa zabilježena su i po godinama istraživanja. Najveći prosječan prinos je evidentiran u 2013. godini (1.292,7 kg ha^{-1}), te je on bio statistički značajno veći od prinosa u 2011. (1.030,3 kg ha^{-1}) i 2012. godini (978,7 kg ha^{-1}). Jedan od razloga zašto je heljda ostvarila najbolji prinos u 2013. godini su relativno povoljniji vremenski uslovi, to jest najveći prinos je zabilježen u godini sa relativno većim i ravnomjernijim rasporedom oborina u toku vegetacije (tab. 1).
Rezultati istraživanja prezentirani u tabeli 3 pokazuju da je sadržaj fenola značajno zavisio od godine istraživanja. U 2012. godini (3,67 mg GAE g^{-1} SM) sve varijante sjetve imale su visok sadržaj fenola, te je on u prosjeku bio za oko deset puta veći u odnosu na 2011. godinu (0,37 mg GAE g^{-1} SM). Visok sadržaj je zabilježen u godini u kojoj je tokom vegetacije evidentirana niža količina oborina i viša srednja mjesečna temperatura. Može se pretpostaviti da su vremenski uslovi uzrok nastalih razlika. Značajan uticaj temperature i vlage na sadržaj fenola zabilježen je i u radu Ghimeray i sar. (2009); Lumingkewas i sar. (2015); Kreft i sar. (2013); Kalinová i Dadáková (2004) i dr. Trogodišnji prosjek pokazuje da je unutar vrste bilo oscilacija u sadržaju ukupnih fenola uzrokovanih varijantom norme sjetve, ali one nisu bile statistički značajne. Najniži sadržaj je zabilježen u varijanti ogleda N1, a najviši u varijanti N3.

ZAKLJUČCI

Rezultati istraživanja dobiveni u okviru poljskih istraživanja ukazuju da povećanje norme sjetve uzrokuje povećanje prinosa, te bi sjetvu obične heljde (sorta darja) u kontinentalnim uslovima Bosne i Hercegovine trebalo obavljati sa normom ne manjom od 100 kg ha^{-1}.
Najveći uticaj na sadržaj fenola u zrnu su imali vremenski uslovi, te je proizvodnju heljde sa povišenim sadržajem fenola moguće ostvariti izborom lokaliteta uzgoja sa toplim i suhim podnebljem.

LITERATURA

Alekseyeva, O. S., Krynytska, L. A., Ross, V. I. (2001): Identifying Optimum Seeded Time for Buckwheat in Rice Crop-Rotation in Ukraine. Proc. 8th int. Symp. of Buckwheat. Chunchon, Koreja.

Bohle, M., Karow, R., Marx, E., James, S. (1998): Seeding rate effect on winter triticale and soft white winter wheat: Year II, 1998: Oregon State University, Central Oregon. Agricultural Research Center Annual Report for 1998, Report No. 1003.

Bystrická, J., Vollmannová, A., Margitanová, E., Čičová, I. (2010): Dynamics of polyphenolic formation in different plant parts and different growth phases of selected buckwheat cultivars. Acta Agric. Slov., 95 (3): 225-229.

Choio, Y. S., Lee, B. H., Shin, T. H., Lee, H. H. (2001): Hypotensive effect of dietary buckwheat in SHR. Proc. 8th int. Symp. Buckwheat. Chunchon, Korea.

Christa, K., Soral-Śmietana, M. (2008): Buckwheat Grains and Buckwheat Products – Nutritional and Prophylactic Value of their Components – a Review. Czech J. Food Sci. 26 (3): 153–162.

Gadžo, Drena, Đikić, Mirha, Muminović, Š., Hadžić, Azra (2007): Mogućnost proizvodnje heljde u Bosni i Hercegovini. Zbornik sažetaka 42. hrvatskog i 2. međunarodnog simpozija agronoma. Opatija, Hrvatska.

Gadžo, Drena, Đikić, Mirha, Oručević Žuljević, Sanja, Gavrić, T., Grahić, J. (2016): Proizvodnja heljde u brdsko-planinskim područjima – dosadašnja iskustva i budući izazovi. Unapređenje poljoprivrede, šumarstva i vodoprivrede u kraškim, brdskim i planinskim područjima – racionalno korištenje i zaštita, Posebna izdanja ANUBiH CLXIX, OPMN DOI: 10.5644/PI2017.169.05. Sarajevo, BiH. 26: 51-60.

Germ, Mateja (2004): Environmental Factors Stimulate Synthesis of Protective Substancesin Buckwheat. Proc. 9th int. Symp. Buckwheat, Prag, Czech Republic.

Ghimeray, A. K., Sharma, P., Briatria, X. (2009): Phenolic Content and Free Radical Scavenging Activity of Seed, Seedling and Sprout of Buckwheat. Proc. 11th int. Symp. Buckwheat. South Korea.

Glamočlija, Đ., Ćosić, Z., Dražić, S., Ikanović, J., Milutinović, M., Đokić, J. (2012): Uticaj agroekoloških i zemljišnih uslova na morfološke osobine i prinos heljde. Zbornik naučnih radova Instituta PKB Agroekonomik 18(1-2): 71-77.

Guo, X. D., Ma, Y. J., Parry, J., Gao, J. M., Yu, L. L., Wang, M. (2011): Phenolics Content and Antioxidant Activity of Tartary Buckwheat from Different Locations. Molecules, 16: 9850-9867.

Ikanović, J., Rakić, S., Popović, V., Janković, S., Glamočlija, Đ., Kuzevski, J. (2013): Agroekološki uslovi gajenja i morfološko-produktivna svojstva heljde. Biotechnology in Animal Husbandry. 29(3): 555-562.

Kalinová, J., Dadakova, E. (2004): Varietal Differences of Rutin in Common Buckwheat (*Fagopyrum esculentum* Moench) Determined by Micellar Electrokinetic Capillary Chromatography. Proc. 9th int. Symp. Buckwheat, Prag, Češka.

Kawa, J. M., Taylor, C. G., Przybylski, R. (2003): Buckwheat concentrate reduces serum glucose in streptozotocin-diabetic rats. Journal Agric. Food Chemistry. 51(25).

Kreft, S., Janeš, D., Kreft, I. (2013): The content of fagopyrin and polyphenols in common and tartary buckwheat sprouts. Acta Pharm. 63:1-8.

Kvashchuk, E. V. (1992): Buckwheat Crop Formation and Planting Density. Proc. 5th Int. Symp. Buckwheat. Taiyuan, Kina.
Kwang, J. C., Park, J. I., Park, B. J., Hong, S. K., Lim, Y. S. Park, C. H. (2007): Effects of Planting Density and Fertilization on Yield and Rutin Content in Tartary Buckwheat. Proc. 10th int. Symp. Buckwheat. Yangling, Kina. 329-333.
Li, J., Gong, F., Li, F. (2016): Hypoglycemic and hypolipidemic effects of flavonoids from tatary buckwheat in type 2 diabetic rats. Biomedical Research. 27(1): 132-137.
Lumingkewas, A., Koesmaryono, Y., Aziz Impron, S., Sugimoto, H. (2015): The Influence of Temperature to Rutin Concentration of Buckwheat Grains in Humid Tropic. IJSBAR 20(1): 1-9.
Maletić, Radojka, Jevđović, R. (2003): The influence of meteorological conditions on major quantitative and qualitative traits of buckwheat (*Fagopyrum esculentum* Moench). Journal of Agricultural Sciences 48(1): 11-19
Oomah, B. D., Campbell, G. C., Mazza, G. (1996): Effects of cultivar and environment on phenolic acids in buckwheat. Euphytica 90: 73–77.
Park, B. D., Park, C. H. (2004): Cytotoxic activities of Tartary buckwheat against human cancer cells. Proc. 9th int. Symp. Buckwheat. Prag, Češka.
Sakač, Marijana, Sedej, Ivana, Mandić, Anamarija, Mišan, Aleksandra (2014): Antioksidativna svojstva brašna od heljde – doprinos funkcionalnosti pekarskih, testeničarskih i brašneno-konditorskih proizvoda. Hemijska industrija 5-6 (60).
Sheng, H. T., Sheng, H. Ch. (1992): Studies on increasing grain yield of buckwheat in Taiwan. Bulletin of TDAIS, 35: 1-10.
Tomotake, H., Shimaoka, I., Kayashita, J., Nakajoh, M., Kato, N. (2001a): Buckwheat protein suppress plasma cholesterol more strongly then soy protein isolate in rats by enhancing fecal excretion of steroids. Proc. 8th int. Symp. Buckwheat. Chunchon, Koreja. 587-594.
Tomotake, H., Liu, Z., Huang, X., Ishikawa, W., Kayashita, J., Kato, N. (2001b): Feeding buckwheat protein suppresses 1,2-Dimethylhydrazine induced colon carcinogenesis in rats. Proc. 8th int. Symp. Buckwheat. Chunchon, Koreja. 259-601.
Varga, B., Svčnjak, Z., Pospišil, Ana, Vinter, J. (2000): Promjene nekih agronomskih svojstava sorata ozime pšenice u ovisnosti o razini agrotehnike. ACS, Agric. conspec. sci. 65(1): 37-44.
Vilcāns, M., Gaile, Z. (2013): Influence of sowing type, time and seeding rates on the buckwheat (Fagopyrum esculentum) yield quality. Research for Rural Development - International Scientific Confer. 1.
Wang, J., Liu, Z., Fu, X., Run, M. (1992): A clinical observation on the hypoglemic effect of Xinjiang buckwheat. Proc. 5th Int. Symp. Buckwheat. Taiyuan, Kina.
Wang, Z., Liu Anlin, Ren Shuhua (1986): Study on the high-yieldlng cultivation of Buckwheat. Proc. 3th int. Symp. Buckwheat. Pulawy, Poljska. 79-94.

A PRELIMINARY STUDY ON THE EFFECT OF DIFFERENT IRRIGATION REGIMES AND POTASSIUM LEVELS ON THE GRAIN YIELD AND SOME YIELD CHARACTERISTICS OF SWEET SORGHUM (*Sorghum bicolor* (L.) Moench *var. saccharatum*)*

Hakan Geren[1], Aleksandar Simić[2], Tuğçe Özdoğan[1], Ivan Krga[2]

Original scientific paper

Summary

A pot study was carried out to determine the effect of different irrigation regimes and potassium levels on the grain yield and some other yield components of sweet sorghum (*Sorghum bicolor* (L.) Moench *var. saccharatum*) (cv. Sugar drip) under outdoor conditions in 2015. Four different potassium levels (0, 50, 100, 150 kg K_2O ha^{-1}) and five irrigation treatments were applied in the experiment. The first treatment was 100% of the field capacity (FC) as a control, and, the others received 80%, 60%, 40% and 20% of the FC, respectively as deficit irrigation treatments. The results showed that K application and water stress had significant effects on the grain yield and yield components. Reducing the yield components, especially the panicle length per plant and harvest index, which reduced the grain yield per plant, stimulated drought stress. The greatest yield was obtained at full irrigation (100% of FC). Potassium increased average grain and biological yield by 342% and 143%, respectively compared to the control (0 kg K ha^{-1}) through improving growth conditions.

Keywords: *sweet sorghum, potassium levels, deficit irrigation, grain yield.*

INTRODUCTION

Drought is a significant limiting factor for agricultural productivity and generally inhibits plant growth through reduced water absorption and nutrient uptake. Potassium is a valuable nutrient in decreasing the effects of water stress for the survival of crop plants. Sweet sorghum (*Sorghum bicolor* L. Moench) is one of the 5 major cultivated species in the world, in terms of production and acreage. It is consumed as food and feed (Almodares *et al*., 2007a) and used for sugar, ethanol and paper pulp production (Berenji and Dahlberg, 2004; Gnansounoua *et al*., 2005). In Serbian conditions mainly forage sorghum and Sudan grass are produced (Ćupina *et al*., 2007). This species is well adapted to sub-tropical and temperate regions. It is highly

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina

[1] University of Ege, Faculty of Agriculture, Izmir-Turkey
[2] University of Belgrade, Faculty of Agriculture, Belgrade-Serbia
Correspondence: Hakan Geren, hakan.geren@ege.edu.tr

biomass productive and water efficient, resistant to drought and salinity, and has a remarkable yield potential even in marginal environments (Steduto *et al*., 1997; Amaducci *et al*., 2004).

Under low soil moisture conditions, sorghum maintains its physiological activity close to that of plants with sufficient moisture by increasing root length, density, and water-use efficiency. This is supported by the fact that sorghum is able to maintain its physiological activity even under drought stress, compared to well-watered sorghum plants (Zegada-Lizarazu *et al*., 2012). Better adaptation to water deficiency in sorghum, compared to maize can also be observed. This is due to; aforementioned, better physiological activity and enlarged root system when under water deficiency stress (Zegada-Lizarazu *et al*., 2012). On the other hand, root water uptake efficiency in drought stressed maize was sustained close to its potential, but not in drought stressed sweet sorghum (Zegada-Lizarazu *et al*., 2012). Other than water regime, crop nutrition has a high effect on the sorghum productivity and quality. One of the mechanisms for improving plant tolerance to drought is to apply K, which seems to have a beneficial effect in overcoming soil moisture stress (Zareian *et al*., 2013). Increased application of potassium has been shown to enhance photosynthetic rate, plant growth, yield and drought resistance in different crops under water stress conditions (Sharma *et al*., 1996; Tiwari *et al*., 1998; Yadav *et al*., 1999; Egilla *et al*., 2001). It have been suggested that accumulation of K^+ by plants before initiation is a good strategy to allow the plant to survive a sudden environmental abiotic stress (Kafkafi, 1990). In addition, potassium has the potential to increase various yield parameters such as stem fresh weight, total fresh weight, total sugar, and juice extract (Almodares *et al*., 2008). The objective of the study was to evaluate the responses to drought stresses under different potassium levels of sweet sorghum determining grain yield and some other yield characteristics.

MATERIALS AND METHODS

A variety 'Sugar drip' of sweet sorghum (*Sorghum bicolor* (L.) Moench *var. saccharatum*) was grown outdoors in a pot, of 24-cm diameter and at 40-cm depth filled by commercial field soil (17 kg), in an experimental area of Field Crops Department, the Faculty of Agriculture, University of Ege, Izmir/Turkey during the 2015 growing season from July to October. Some meteorological data from the experimental area in Bornova-Izmir and some soil characteristics of the experimental field soil are presented Table 1 and Table 2, respectively. There were no limiting factors to grow sorghum crops with regard to climate and soil properties, and the crops have been successfully cultivated with the support of irrigation and fertilization.

Table 1. Some meteorological data of experimental area in Bornova in 2015

	Temperature (°C)		Precipitation (mm)		Relative humidity (%)	
Months	2015	LYA	2015	LYA	2015	LYA
July	28.0	28.3	1.8	2.1	45.2	51.2
August	27.7	27.9	2.6	1.7	39.5	53.9
September	23.7	23.9	15.0	19.9	55.4	58.0
October	18.8	19.1	45.3	43.2	59.5	64.0
✕ - Σ	24.6	24.8	64.7	66.9	49.9	56.8

LYA: Long year's average, ✕: mean, Σ: total

Table 2. Some soil characteristics of the experimental field soil

Sand (%)	80.2	pH	6.71
Clay (%)	1.7	OM (%)	1.2
Silt (%)	18.1	N (%)	0.1
$CaCO_3$ (ppm)	1250	P (ppm)	0.7
Salt (%)	0.03	K (ppm)	15

Factorial design with three replications was assigned. Seeds of sweet sorghum were sown with different fertilization levels of potassium (0, 50, 100, 150 kg K_2O ha^{-1}), on 4^{th} July 2015 at 1- to 2- cm depth in plastic pots. However, nitrogen was added to all treatments at a rate of 150 kg N ha^{-1} and also phosphorus at a rate of 100 kg P_2O_5 ha^{-1} (except K0). Nitrogen fertilizer was applied in two equal doses. ½ rate of N (urea) and full doses of K (potassium sulphate) or P (triple superphosphate) was mixed with soil and supplied as a single application. The other ½ rate of N (ammonium nitrate) was added when the crops were at approximately 40 cm tall. When the first 2 or 3 leaves emerged, a hand-thinning was carried out to give a seedling rate of two plants per pot.

Five irrigation treatments were applied in the experiment. The first treatment was 100% of the field capacity (FC, %) as a control. The other treatments received 80%, 60%, 40% and 20% of the FC, respectively as deficit irrigation treatments. The deficit irrigation treatments were applied after two weeks from cultivation in the pots and continued till the end of the experiment. During the experiment, all of the pots were weighed daily, and the amount of water lost was replaced by tap water to maintain the soil water content. In possible rainy weather, a nylon sheet covered the experiment. Weeds were manually removed from the pots; no herbicide was used to control weeds. Alphacypermethrin was applied at 6-7 leaf stage of crops to control European corn borer (*Ostrinia nubilalis*). Panicles in every pot were isolated with a net to avoid bird damage and obtain grain yield.

The plants were harvested when the sweet sorghum had reached physiological maturity of seeds (~12% moisture). Harvested plant were threshed and weighed to determine grain yield. Every plant in each pot was used for the measurements set out below: plant height (cm); the plant was measured from the soil surface to the top level of the plant before harvest. The harvest index (%) was calculated as the grain yield per plant over the total dry mass, above ground biomass per plant at harvest (g/g). Sorghum grains (100) were weighted and converted for calculation of 1000 grain weights. At the end of the experiment, the soil was gently washed from roots, and the roots were oven dried at 70°C until they reached a constant mass to measure the dry weight. Grain samples were ground to a fine powder and taken for determination of the crude protein (CP) content (Kjeldahl $N_{\%}$ x 6.25).

All data were statistically analyzed using analysis of variance (ANOVA) with the Statistical Analysis System (SAS, 1998). Probabilities equal to or less than 0.01 were considered significant. If ANOVA indicated differences between treatment means, a LSD test was performed to separate them (Stell *et al.*, 1997).

RESULTS AND DISCUSSION

Meteorological conditions during experimental period were similar to long year's average (Table 1). Also, soil characteristics were appropriate for irrigation (sandy soil), with low OM, N, P and K content (Table 2), which justify addition of macronutrients in the experiment.

Table 3. Effect of different irrigation regimes and potassium levels on the grain yield and some yield characteristics of sweet sorghum.

K levels	K0	K50	K100	K150	Mean	K0	K50	K100	K150	Mean
Irrigation	--------- Plant height (cm) ---------					--------- Panicle length (cm) ---------				
FC-100%	161.3	185.0	221.0	222.1	197.4	15.6	20.5	22.1	22.7	20.2
FC-80%	159.3	183.7	220.3	223.1	196.6	12.7	19.5	22.7	23.2	19.5
FC-60%	151.7	171.7	211.0	217.7	188.0	10.7	16.1	17.5	19.5	16.0
FC-40%	124.3	152.3	174.7	181.4	158.2	7.6	15.2	16.2	17.2	14.1
FC-20%	99.5	127.4	147.7	155.0	132.4	4.9	7.9	8.7	10.8	8.1
Mean	139.2	164.0	194.9	199.9	174.5	10.3	15.8	17.4	18.7	15.6
LSD (1%)	K:2.7 I:3.0 int:6.0 CV(%):1.56					K:0.8 I:0.9 int:1.8 CV(%):5.37				
	--------- Biological yield (g plant^{-1}) ---------					--------- Harvest index (%) ---------				
FC-100%	152.7	166.0	197.4	206.8	180.7	12.2	20.8	24.4	25.0	20.6
FC-80%	143.3	167.6	203.4	209.4	180.9	10.7	17.6	22.6	24.2	18.8
FC-60%	142.0	156.8	194.4	200.1	173.3	10.4	16.9	21.3	23.8	18.1
FC-40%	122.3	127.7	155.1	176.4	145.4	8.5	18.2	20.4	20.7	17.0
FC-20%	99.7	118.6	132.8	155.8	126.7	4.5	8.9	18.2	19.9	12.9
Mean	132.0	147.3	176.6	189.7	161.4	9.3	16.5	21.4	22.7	17.5
LSD (1%)	K:3.9 I:4.4 int:8.8 CV(%):2.47					K:1.0 I:1.1 int:2.3 CV(%):5.85				
	--------- 1000 grain weight (g) ---------					--------- Grain yield (g plant^{-1}) ---------				
FC-100%	19.4	20.6	23.9	23.9	21.9	18.7	34.5	48.2	51.6	38.2
FC-80%	20.3	20.9	24.0	25.8	22.8	15.3	29.4	46.1	50.7	35.4
FC-60%	21.1	22.3	24.1	26.6	23.5	14.7	26.5	41.4	47.7	32.6
FC-40%	21.7	23.5	25.6	26.8	24.4	10.4	23.3	31.5	36.6	25.4
FC-20%	22.1	26.0	25.7	27.2	25.2	4.5	10.6	24.2	31.1	17.6
Mean	20.9	22.7	24.7	26.1	23.6	12.7	24.8	38.3	43.5	29.8
LSD (1%)	K:1.4 I:1.6 int: ns CV(%):6.24					K:1.3 I:1.5 int:2.9 CV(%):4.53				
	--------- Dry root weight (g plant^{-1}) ---------					------- Grain crude protein content (%) --------				
FC-100%	72.3	98.3	120.0	134.3	106.3	7.7	8.2	8.8	8.8	8.4
FC-80%	62.3	93.0	121.7	137.7	103.7	6.9	8.0	8.6	8.8	8.1
FC-60%	62.0	92.9	119.2	133.2	101.8	6.5	7.1	8.0	8.2	7.4
FC-40%	52.0	79.0	88.7	102.7	80.6	5.8	6.0	6.4	6.6	6.2
FC-20%	33.2	73.0	81.7	93.0	70.2	5.7	5.8	6.0	6.1	5.9
Mean	56.4	87.2	106.2	120.2	92.5	6.5	7.0	7.5	7.7	7.2
LSD (1%)	K:2.9 I:3.3 int:6.5 CV(%):3.18					K:0.3 I:0.3 int:0.6 CV(%):3.99				

K: potassium level, I: irrigation level, int: interaction, CV(%): coefficient of variation, ns: not significant.

Plant height showed highest values when irrigation was 100% FC (197.4 cm) and 80% FC (196.6 cm) when fertilized with 150 kg K ha^{-1} (Table 3). In a similar experiment, plant height had highest values when irrigation was 70% FC (140.4 cm), 50% FC (118.1 cm), 30% FC (94.4 cm) (Asgharipour and Heidari, 2011). For the same amounts of

potassium (150 kg ha^{-1}), plant height had average values of 199.9 cm in our experiment, compared to 119.9 cm (Asgharipour and Heidari, 2011).
Same trend has been observed in panicle length (Table 3). Highest values were recorded when FC was 100% (20.2 cm) and 80% (19.5 cm) and 150 kg ha^{-1} of potassium had been used (18.7 cm). Compared to other findings, highest values were also recorded when highest irrigation quantities had been used (70%), while level of potassium did not have any significant effect (Asgharipour and Heidari, 2011).
Biological yield had highest values when irrigation was 100% and 80% FC and when 150 kg of potassium ha^{-1} was applied. It is easy to conclude, speaking of plant height, panicle length and biological yield, and harvest index, that values have growing trend when observing from the lowest to the highest quantities of applied irrigation and K ha^{-1} (Table 3). In a similar experiment, when 4 levels of potassium were applied, the highest biological yields were recorded at the highest doses of applied potassium (200 kg K ha^{-1} – 14.37 t ha^{-1}; 250 kg K ha^{-1} – 13.87 t ha^{-1}), and applied irrigation (FC 70% - 14.46 t ha^{-1}) (Asgharipour and Heidari, 2011). It is expected and shown that yield of sorghum might be affected by quantities of water applied, but it has also been recorded that some sorghum cultivars might be affected by water quality as well (Almodares *et al*., 2007b). In Serbian conditions, on the calcareous chernozem soil on the loess terrace, the highest yield of forage sorghum was achieved with soil moisture 70-75% FC (95.74 t ha^{-1} fresh biomass), and that value represents the technical minimum or the lower limit of optimum soil moisture for forage sorghum in the soil with medium and heavy mechanical composition (Pejić *et al*., 2005)
Grain weight was not differently affected by quantities of 150 kg K ha^{-1} (26.1 g 1000 grains^{-1}) and 100 K kg ha^{-1} (24.7 g 1000 grains^{-1}), but values were significantly lower when 50 kg was applied (22.7 g 1000 grains^{-1}) and in the control (20.9 g 1000 grains^{-1}). Highest grain weight values were recorded when FC was 20% (25.2 g 1000 grains^{-1}), and lowest when FC was 100% (21.9 g 1000 grains^{-1}). There is a mismatch between our results and results in other studies where highest values of grain weight were recorded when FC was also highest (FC 30% - 2.13 g 100 grains^{-1}; FC 50% - 2.30 g 100 grains^{-1}; FC 70% - 2.50 g 100 grains^{-1}) (Asgharipour and Heidari, 2011).
Grain yield, dry root weight and grain crude protein content also increased as the quantities of applied potassium and irrigation were increased, thus the highest values were obtained when FC was 80-100% and potassium was 100-150 kg ha^{-1}. It is useful to mention that all examined characteristics and parameters of the yield increased when the application of two examined factors was increased, except for the weight of 1000 grain, where it has been found that high levels of water tend to decrease the weight. Many researchers (Asgharipour and Heidari, 2011; Dahmardeh *et al.*, 2015) suggested that potassium application could have positive effects on the yield of sorghum and water use efficiency only in the stress less or justified stress conditions.
Overall, potassium treatments showed a clearer, more substantial, in comparison to water treatments. In all examined yield characteristics, the lowest effect was on the treatment K0 (without K application), while the best results were obtained with high potassium amount (K150).

CONCLUSION

The paper emphasizes the importance of sweet sorghum in the grain yield production. Peculiarities in production technology regarding irrigation regimes and potassium levels are taken into consideration. Considering overall results of the pot experiment, it could be concluded that a deficit in irrigation or water stress significantly decreases plant growth and yield. Using potassium fertilizers in the right way should enable more efficient mitigation of the unfavourable environmental conditions, especially drought stress. Higher potassium fertilization produced significantly higher yield and yield components of sweet sorghum compared to lower K amounts. Also, the greatest plant height, panicle length, biological yield, harvest index, grain yield, dry root weight and grain crude protein content were obtained at full irrigation (100% of FC), while the deficit treatments (especially 40% and 20% of the FC) had a positive effect on the 1000 grain weight.

REFERENCES

Almodares, A., Taheri, R., Adeli, S. (2007a): Inter-relationship between growth analysis and carbohydrate contents of sweet sorghum cultivars and lines. J. Environ. Biol., 28: 527-531.

Almodares, A., Sharif, M. E. (2007b): Effects of irrigation water qualities on biomass and sugar contents of sugar beet and sweet sorghum cultivars. J. Environ. Biol., 28(2): 213-218.

Almodares, A., Taheri, R., Chung, I. M., Fathi, M. (2008): The effect of nitrogen and potassium fertilizers on growth parameters and carbohydrate contents of sweet sorghum cultivars. J. Environ. Biol., 29(6): 849-852.

Amaducci, S., Monti, A., Venturi G. (2004): Non-structural carbohydrates and fibre components in sweet and fibre sorghum as affected by low and normal input techniques. Ind. Crops Products, 20: 111-118.

Asgharipour, M. R., Heidari, M. (2011): Effect of potassium supply on drought resistance in sorghum: Plant growth and macronutrient content. Pak. J. Agri. Sci., 48(3): 197-204.

Berenji, J., Dahlberg J. (2004): Perspectives of sorghum in Europe. J. Agron. Crop Sci., 190: 332-338.

Ćupina, B., Pejić, B., Erić, P., Krstić, Đ., Vučković, S. (2007): Particularities in agronomy of forage sorghum and Sudan grass in agro-ecological conditions of Vojvodina province (in Serbian). Zbornik radova Instituta za ratarstvo i povrtarstvo/ Field and Vegetable Crops Research, 44, (1): 291-300.

Dahmardeh, K., Rad, M. R. P., Rad, M. R. N. and Hadizadeh, M. (2015): Effects of potassium rates and irrigation regimes on the yield of forage sorghum in arid regions, International Journal of Agronomy and Agricultural Research, 6(4):207-212.

Egilla, J. N.; Davies, F. T. Jr. and Drew, M. C. (2001): Effect of potassium on drought resistance of *Hibiscus rosa-sinensis* cv. Leprechaun: Plant growth, leaf macro and micronutrient content and root longevity. Plant and Soil 229(2): 213- 224.

Gnansounoua, E., Dauriata A., Wyman, C. E. (2005): Refining sweet sorghum to ethanol and sugar: Economic trade-offs in the context of North China. Bioresource Tech., 96, 985-1002.

Kafkafi, U. (1990): The functions of plant K in overcoming environmental stress situations. In: Proc. 22nd colloquium of IPI, 81-93, held in Soligorsk, USSR, IPI, Bern.

Pejić, B., Maksimović, L., Karagić, Đ., Mihailović, V. M. (2005): Yield and evapotranspiration of forage sorghum depending on preirrigation soil moisture (in Serbian). Zbornik radova Instituta za ratarstvo i povrtarstvo/ Field and Vegetable Crops Research, (41):191-198.

SAS Institute (1998): INC SAS/STAT user's guide release 7.0, Cary, NC, USA.

Stell, R. G. D., Torrie, J. A. and Dickey, D. A. (1997): Principles and Procedures of Statistics, A Biometrical Approach 3rd Edi. Mc Graw Hill Book. INC. NY.

Sharma, K. D., Nandwal, A. S., Kuhad, M. S. (1996): Potassium effects on CO_2 exchange, ARA and yield of clusterbean cultivars under water stress. Jour of Potassium Research 12: 412-423.

Steduto, P., N. Katerji, H. Puertos-Molina, M. Ünlü, M. Mastrorilli and G. Rana (1997): Water-use efficiency of sweet sorghum under water stress conditions gas-exchange investigations at leaf and canopy scales. Field Crops Res., 54: 221-234.

Tiwari, H. S.; Agarwal, R. M. and Bhatt, R. K. (1998): Photosynthesis, stomatal resistance and related characters as influenced by potassium under normal water supply and water stress conditions in rice (*Oryza sativa* L.). Indian Journal of Plant Physiology 3(4): 314-316.

Yadav, D. S., Goyal, A. K. and Vats, B. K. (1999): Effect of potassium in *Eleusine coracana* (L.) Gaertn. under moisture stress conditions. Journal of Potassium Research, 15(1-4): 131-134.

Zareian, A., Abad, H., Hamidi, A., Mohammadi, G., Tabatabaei, S. (2013): Effect of drought stress and potassium foliar application on some physiological indices of three wheat (*Triticum aestivum* L.) cultivars. Annals of Biological Research. 4(5): 71-74.

Zegada-Lizarazu, W., Zatta, A., Monti, A. (2012): Water uptake efficiency and above- and belowground biomass development of sweet sorghum and maize under different water regimes. Plant Soil, 351(1): 47-60.

BIOMASS YIELD AND ETHANOL PRODUCTION CAPACITY OF SWEET SORGHUM (*Sorghum bicolor var. saccharatum*) CULTIVARS AFFECTED BY HARVEST STAGES*

Hakan Geren[1], Behçet Kır[1], Yaşar Tuncer Kavut[1]

Original scientific paper

Summary

This study was conducted in order to determine the adaptability, biomass yield and bio-ethanol production capacity of sweet sorghum. Sorghum was grown in summer period as a second crop on the experimental fields of Faculty of Agriculture, Ege University under Mediterranean ecological conditions of Bornova-Izmir during two years in 2013-2014. Two different sweet sorghum cultivars (cv. Keller and Rio) were used as crop material. Sweet sorghums were cut in three different harvesting stages (panicle emergence, anthesis and doughy). Some traits were tested in the experiment such as fresh biomass and stalk yield, sugar content and yield, syrup and ethanol yields. Results indicated that there were significant differences between harvest stages and sweet sorghum cultivars in terms of fresh biomass yield and ethanol production capacities. Delaying harvest stage affected positively biomass and ethanol yields. It was also concluded that cv. Keller was superior to cv. Rio with regard to above mentioned traits.

Keywords: *sweet sorghum, cultivar, harvesting stage, biomass yield, ethanol production.*

INTRODUCTION

Today, food and energy needs are becoming more important due to the growing world population. In particular, all countries are determining their strategies towards the food and energy sectors. Large amounts of funds are allocated to research and development in this area, and scientists are searching for solutions to the problem. The future of humanity depends on finding solutions to these two problems. It is considered that there is often an energy problem based on international disputes, one of the alternative solutions is the production of vegetable ethanol, which is already starting to be consumed with fossil fuels (Hugar *et al.*, 2010; Lizarazu and Monti, 2015). Sweet sorghum can also serve special purposes due to its stalk juice, which has rich sugar content such as sugar cane. In addition, the adaptation capability of the sweet sorghum

---

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] University of Ege, Faculty of Agriculture, Department of Field Crops, Izmir-Turkey
Correspondence: Hakan Geren, hakan.geren@ege.edu.tr

is wider than other sorghums. Many researchers report that sweet sorghum cultivation is more rational because it requires less water than plants such as sugar cane, corn or sugar beet (Ricaud and Arceneaux, 1990; Ryan and Spencer, 2001; Reddy and Sanjana, 2003).

A number of investigators have reported that sweet sorghum stalks are mechanically extracted and contains 16-23% sugar in stalk juice, with sucrose being the predominant disaccharide, depending on the varietal characteristics, syrup and bioethanol are produced (Gibbons *et al.*, 1986; Borade *et al.*, 2015; Oyier *et al.*, 2017). Quantity and quality of sugar in stem of sweet sorghum have been changed at different growth stages, so harvesting stage is an important factor on sugar content (Almodares *et al.*, 2008, 2010). In addition, sweet sorghum accumulates large amounts of sugar in its stem near the time of grain maturity. Borade *et al.* (2015) reported that the growth stage at 45 days after 50% flowering was found better for maximum sugar contents and yields for sweet sorghum. As it well known that selecting a sweet sorghum cultivar that is well suited to the ecology enables the crop to move through its lifecycle efficiently in a way that best matches its environment. Knowing how a cultivar will work within a specific environment help strike a balance that will aid in procuring the highest potential yield for the crop (Xuan *et al.*, 2015). The aim of this study is to investigate the effects of three different harvesting stages on biomass and ethanol yield and some yield characteristics in two different sweet sorghum cultivars grown as second crops under Bornova ecological conditions in typical Mediterranean climate.

MATERIALS AND METHODS

The field experiment was carried out for two growing seasons (2013-2014) at Bornova experimental fields of Agricultural Faculty of Ege University, Izmir, Turkey, at about 20 m above sea level with typical Mediterranean climate characteristics (Table 1). Some physical and chemical characteristics of experimental soil presented in Table 2. There was no element that restricts the cultivation of sweet sorghum in terms of climate and soil characteristics of the research area, and the crops were successfully cultivated with the irrigation.

Table 1: Some meteorological characteristics of experimental area in Bornova in 2013 and 2014

	Temperature (°C)			Total precipitation (mm)		
Months	2013	2014	LYA	2013	2014	LYA
Jan.	9.4	9.9	8.1	252.5	133.8	109.7
Feb.	11.2	9.7	8.6	187.0	45.6	89.8
March	14.0	11.5	10.8	56.8	108.4	72.3
April	17.3	15.0	15.0	30.2	76.8	48.9
May	22.7	19.3	20.2	43.7	2.2	32.2
June	25.7	23.8	25.0	27.1	75.2	8.2
July	28.4	26.8	27.6	0.0	16.0	3.6
August	28.7	28.3	27.0	20.2	6.0	2.1
Sept.	24.0	23.0	22.2	5.1	18.6	17.0
Oct.	17.2	18.8	18.0	94.1	49.1	46.8
Nov.	15.0	13.2	13.2	128.9	15.2	80.3
Dec.	8.5	11.1	9.9	9.1	206.8	122.3
$\bar{X}$ - Σ	18.5	17.5	17.1	854.7	753.6	633.2

LYA: Long year average, $\bar{X}$: mean, Σ:total

Table 2: Some physical and chemical characteristics of experimental soil

Characteristics	0-20 cm	20-40 cm
Sand (%)	24.72	32.72
Clay (%)	32.56	30.56
Silt (%)	42.72	36.72
Texture	Clay loam	Loam
pH	8.2	7.8
$CaCO_3$ (%)	21.52	18.64
Total salt (%)	0.095	0.075
Organic mat. (%)	1.132	1.151
Total N (%)	0.101	0.123
Available P (ppm)	0.39	0.41
Available K (ppm)	395	297

The experiments were carried out with a randomized complete block design with three replicates. In both years, seeds of sweet sorghum cultivars ‘Keller’ and ‘Rio’ were used as crop material. Two factors have been investigated in this study; *i*) two different sweet sorghum cultivar and *ii*) three different harvest stages (panicle emergence, anthesis and doughy). Plot size was 2.80 m x 5 m (14 m^2), with 4 rows (70 cm row spacing). Seeding was done by hand at the rate of 10 kg ha^{-1} on July 8 in 2013 and 2014, respectively. The rows thinned by hand after emergence to the 25 cm intra-row spacings.

Based on soil test results, sweet sorghum was fertilized with 220 kg N and 100 kg P_2O_5 per hectare. Half a dose of N fertilizer (urea) and full dose of P (triple superphosphate) were applied before sowing, and the rest of nitrogen (NH_4NO_3) was applied when the sweet sorghum plant height was 45-50 cm. Drip irrigation system was installed on the field during the establishment and growing seasons. No herbicide was used to control weeds; only hand hoe was done twice. Alphacypermethrin was applied at 6-7 leaf stage of crops to control *Ostrinia nubilalis*. No evident crop diseases were detected.

Plots were harvested at 3 different stages of sweet sorghum, cutting mid 2 rows of plots in order to avoid border effects (net 7 m^2), by cutting the plants leaving a 5 cm stubble height, during September and October in both years. Harvested fresh crops were weighed and dried to a constant weight at 65ºC during 48 h. In order to determine the yield components 20 stems with attached leaves and heads were randomly selected from each replication and measured individually. Stalks (leafless stem) were weighed after the leaves and panicles were removed by hand, then the stalks were chopped and passed through special press to obtain juice yield. Juice yield was calculated by measuring with graduated measure cylinder. The sugar content (total soluble solids concentration, brix) was measured with a refractometer according to Anonym (2010). The sugar yield was calculated as kg ha^{-1} by multiplying the sugar ratio by the juice yield. The alcohol yield (litre ha^{-1}) was calculated from the theoretical conversion by formula of EtOH=[(total sugar yield/5.68)x3.78]x0.8 (Anonym, 2010).

The collected data for the two years were subjected to analysis of variance (ANOVA) and significant differences between treatment means were determined by using least significant difference (LSD) test at $p \leq 0.05$ level (Stell *et al*., 1997) using a computer software Statistical Analysis System (SAS, 1998).

RESULTS AND DISCUSSION

Fresh biomass yield: The ANOVA results showed that fresh biomass yield of sweet sorghum was significantly affected by the harvest stage-cultivar (HSxC) interaction at 5% probability level (Table 3). Year effect was also significant and average fresh biomass yield of first year (34.71 t ha^{-1}) was lower than second year (36.43 t ha^{-1}). The highest average biomass yield (42.57 t ha^{-1}) was recorded at cv. Keller cut at the doughy stage and the lowest yield (27.52 t ha^{-1}) in cv. Keller harvested at panicle emergence stage.

Table 3: Effect of different harvest stages on yield and some yield parameters of sweet sorghum cultivars

	2013				2014				2 years average			
	I	II	III	Mean	I	II	III	Mean	I	II	III	Mean
Cultivar	Fresh biomass yield (t·ha^{-1})											
Keller	26.95	36.40	41.72	35.02	28.08	39.42	43.42	36.97	27.52	37.91	42.57	36.00
Rio	32.73	33.66	36.81	34.40	34.25	35.17	38.25	35.89	33.49	34.41	37.53	35.15
Mean	29.84	35.03	39.27	34.71	31.17	37.29	40.83	36.43	30.50	36.16	40.05	35.57
LSD	Y:10.4 HS:12.8 C:ns YxHS:ns YxC:ns HSxC:18.1 YxHSxC:ns CV(%):4.3											
	Stalk yield (t·ha^{-1})											
Keller	16.80	24.96	26.95	22.90	17.28	26.28	26.72	23.42	17.04	25.63	26.85	23.17
Rio	10.63	14.64	14.81	13.36	10.46	14.75	15.16	13.45	10.55	14.70	14.99	13.41
Mean	13.71	19.80	20.88	18.13	13.87	20.51	20.94	18.44	13.80	20.17	20.92	18.29
LSD	Y:ns HS:9.0 C:7.4 YxHS:ns YxC:ns HSxC:12.8 YxHSxC:ns CV(%):5.8											
	Total soluble solids concentration (%)											
Keller	8.0	9.8	15.4	11.1	7.5	10.3	16.8	11.5	7.7	10.1	16.1	11.3
Rio	7.8	8.7	12.0	9.5	7.2	9.9	13.2	10.1	7.5	9.3	12.6	9.8
Mean	7.9	9.2	13.7	10.3	7.3	10.1	15.0	10.8	7.6	9.7	14.4	10.5
LSD	Y:0.4 HS:0.5 C:0.4 YxHS:0.7 YxC:ns HSxC:0.7 YxHSxC:ns CV(%):5.7											
	Juice yield (litre·ha^{-1})											
Keller	12480	17320	17390	15730	12760	18210	17370	16110	12620	17760	17380	15920
Rio	7740	9920	9420	9030	7530	9910	9550	9000	7630	9910	9480	9010
Mean	10110	13620	13410	12380	10140	14060	13460	12550	10120	13840	13430	12470
LSD	Y:ns HS:600 C:490 YxHS:ns YxC:ns HSxC:850 YxHSxC:ns CV(%):5.7											
	Sugar yield (kg·ha^{-1})											
Keller	997	1698	2684	1793	952	1883	2917	1918	975	1790	2801	1855
Rio	604	858	1129	864	540	985	1259	928	572	921	1194	896
Mean	801	1278	1907	1328	746	1434	2088	1423	773	1356	1997	1376
LSD	Y:79 HS:97 C:79 YxHS:138 YxC:ns HSxC:138 YxHSxC:ns CV(%):8.4											
	Ethanol yield (litre·ha^{-1})											
Keller	531	904	1429	955	507	1003	1553	1021	519	953	1491	988
Rio	322	457	601	460	288	524	670	494	305	490	636	477
Mean	426	680	1015	707	397	763	1112	757	412	722	1063	732
LSD	Y:43 HS:52 C:43 YxHS:73 YxC:ns HSxC:74 YxHSxC:ns CV(%):8.3											

I: panicle emergence stage, II: anthesis stage, III: doughy stage, Y: year, HS: harvest stage, C: cultivar, ns: not significant

In our study, average results of two years displayed that the fresh biomass yields increased as the harvest time progressed (from panicle emergence to doughy stage) and cv. Keller had higher yield than cv. Rio. These could be due to the growth and development of the crops that was almost completed at doughy stage compared to panicle emergence or anthesis stage. Many researchers (Almodares *et al*., 2006, 2007) stated that fresh biomass yield increases from flowering to physiological maturity stage in sweet sorghum. Almodares *et al.* (2007) investigated three different harvesting stages (flowering, physiological maturity, before chilling) and three different types of sweet sorghum variety (Vespa, IS2325, Rio) under Iranian ecological conditions. They informed that yields increased from 43.83 ton ha^{-1} to 51.89 t ha^{-1} (sugar rate 13.4% to 15.6%) as the harvesting stages progressed, but then the yield decreased to 49.25 t ha^{-1} but that the sugar ratio increased to 16.4%. Genotype differences, higher plant height (not seen in Table 3) and more nodes in cv. Keller than cv. Rio can explain this situation. Our findings were in agreement with some researchers (Almodares *et al*., 2006, 2007).

Stalk yield: In present study, stalk yield of sweet sorghum was significantly affected by the harvest stage-cultivar interaction (Table 3). Year effect was not significant. According to two years average, the highest stalk yield was determined being 26.85 t ha^{-1} in cv. Keller, which was harvested at doughy stage, whereas the lowest yield (10.55 t ha^{-1}) was at panicle emergence stage in cv. Rio. The stalk yield of sweet sorghum increased with delaying harvesting stage in both years. There is diversity of information about stalk yield of sweet sorghum that maturation is an important factor to increase stalk yield. Numerous researcher (Tsuchihashi and Goto, 2004; Reddy *et al*., 2005; Chavan *et al.*, 2009) pointed out that, the accumulated dry matter to stalk by early heading is the determinant factor for the yield capacity of the subsequent growth stage and it is also largely dependent on the climatic conditions of the experimental environments and genotypes. Almodares *et al*. (2007) informed that the effect of harvesting stage on stalk yield of sweet sorghum was significant, and, stalk yield was highest at physiological maturity (51.89 t ha^{-1}) and lowest at flowering (43.83 t ha^{-1}). Some researchers emphasized that average stalk yields of sweet sorghum were from 22.76 to 44.85 t ha^{-1} under the ecological conditions in India (Chavan *et al.*, 2009) and from 21.50 to 26.60 t ha^{-1} in Izmir (Geren *et al.*, 2011), respectively. Tsuchihashi and Goto (2004) stated that there were significant differences on the stalk yield among harvest stages (from 10 days to 60 days after anthesis) of different sweet sorghum cultivars (Wray, Keller, Rio). They also mentioned that stalk yield of Wray and Keller was higher than Rio. In our experiment, cv. Keller had the higher stalk yield at every maturity stage than cv. Rio most probably due to the genotypic differences.

Total soluble solids concentration (sugar content): Sugar rate of sweet sorghum was significantly affected by the YxHS (year-harvest stage) and HSxC (harvest stage-cultivar) interactions in the study (Table 3). Year effect was also significant and average sugar content of second year (10.8%) was slightly higher than the first year (10.3%). Two-year average monitored that the highest sugar content (16.1%) was measured at doughy stage in cv. Keller, and the lowest (7.5%) was at panicle emergence stage in cv. Rio. The harvest of sweet sorghum progressed from panicle emergence to doughy stage, sugar contents had increased due to maturation. Keller had a higher sugar content (1 to 3%) compared to cv. Rio. Many researchers (Tsuchihashi and Goto, 2004; Almodares *et al*., 2007; Chavan *et al*., 2009; Zhao *et al*., 2009) stated that sugar content in sweet sorghum juice had increased as the harvest stages progressed. Our findings seem to confirm by the above-mentioned researcher's results. Almodares *et al*. (2007) reported that in sweet sorghum at physiological maturity stage, sucrose content increased while invert sugar decreased. The authors indicated that at this stage, invert sugars (glucose and fructose) were converted to sucrose, which is in agreement with our results. Therefore, it seems that low sucrose content at flowering could be due high invertase activities, which invert sucrose to glucose and fructose. In contrast, at physiological maturity due to low invertase activity, sucrose does not invert to glucose and fructose. Based on the above results, it seems than cv. Keller is better adapted to experimental area than cv. Rio and it is recommended to plant Keller and harvest at doughy maturity.

Juice yield: Statistical analysis results revealed that juice yield of sweet sorghum in the study was significantly affected by the harvest stage-cultivar interactions (Table 3). Year effect was not significant on this parameter. Two-year average indicated that the lowest juice yield (7630 litre ha^{-1}) was obtained from cv. Rio harvested at the panicle emergence stage, and, the highest juice yield (17760 litre ha^{-1}) was obtained from cv. Keller cut at the anthesis stage. In our experiment, juice yields of sweet sorghum from the panicle emergence to the anthesis stage were increased but later slightly decreased in both years most probably due to decreasing water content of the stalk in doughy stage. Ramesha and Sharanappa (2013) informed that sweet sorghum harvested at maturity recorded significantly higher green biomass yield (31.7 t ha^{-1}) compared to crop harvested at 50% flowering stage and milky stage, but juice yield (9777 litre ha^{-1}) was significantly higher when the crop harvested at milky stage. It was also found that cv. Keller provided higher juice yield than cv. Rio because of Keller's stalk yield was higher in the study. Many researchers stated that there were great differences among the sweet sorghum varieties in terms of juice yield (Clegg *et al.*, 1986; Reddy *et al.*, 2005; Bhairappanavar and Sharanappa, 2011; Geren *et al.*, 2011).

Sugar yield: Calculated sugar yield of sweet sorghum in the study was significantly affected by the YxHS and HSxC interactions (Table 3). Year effect was also significant and average sugar yield of second year (1423 kg ha^{-1}) was higher than the first year (1328 kg ha^{-1}). Two-years average has shown that the highest sugar yield (2801 kg ha^{-1}) was calculated at doughy stage in cv. Keller, and the lowest (572 kg ha^{-1}) was at panicle emergence stage in cv. Rio. It has been found that average sugar yields raised by increasing crop maturity in our study. On this raising, the increase in the sugar content of the juice is more effective. Ramesha and Sharanappa (2013) pointed out that sorghum harvested at maturity recorded significantly higher brix (14.8%) and calculated sugar yield (137 kg ha^{-1}) compared to crop harvested at 50% flowering stage and milky stage. Two times more sugar yield was obtained from cv. Keller than cv. Rio. Geren *et al.* (2011) informed that sugar yields of cv. Keller grown as second crop under Bornova ecology were varied between 1340-1780 kg per hectare. Many researchers have reported that the calculated sugar yields of different sweet sorghum varieties can vary.

Ethanol yield: Theoretic ethanol yield of sweet sorghum in the study was significantly affected by the YxHS and HSxC interactions (Table 3). Year effect was also significant and average ethanol yield of second year (757 litre ha^{-1}) was slightly higher than the first year (707 litre ha^{-1}). Two-year average results indicated that the highest ethanol yield (1491 litre ha^{-1}) was calculated at doughy stage in cv. Keller, and the lowest (305 litre ha^{-1}) was at panicle emergence stage in cv. Rio. Generally, similar results of sugar yield were also determined on ethanol yield in our study. Theoretical ethanol yield of sweet sorghum increased as harvesting stages delayed. Ramesha and Sharanappa (2013) reported that the crop harvested at maturity recorded significantly higher brix (14.8%) and millable stalk (29 t ha^{-1}) compared to crop harvested at 50% flowering stage and milky stage, but ethanol yield (475 litre ha^{-1}) were significantly higher when the crop harvested at milky stage. They also stated that the cultivar "NSSH-1" harvested at milky stage recorded significantly higher juice (11415 litre ha^{-1}) and ethanol yield (591 litre ha^{-1}). Three sweet sorghum cultivars (Madhura, SSV-84 and RSSV-9) were evaluated by Bhairappanavar and Sharanappa (2011) for ethanol production potential under two spacings (45x10 cm, 45x15 cm) and two stages of harvest (50% flowering and at physiological maturity) in Kathalagere, India. They informed that among the cultivars tested 'Madhura' produced significantly higher ethanol yield (556 litre ha^{-1} and 572 litre ha^{-1}) when compared with 'SSV-84' (378 litre ha^{-1} and 405 litre ha^{-1}) and 'RSSV-9' (368 litre ha^{-1} and 426 litre ha^{-1}). In addition, 'Madhura' harvested at physiological maturity showed significantly higher ethanol yield 929 litre ha^{-1} and 958 litre ha^{-1} when compared with that harvested at 50% flowering (183 litre ha^{-1} and 185 litre ha^{-1}). In our study, cv. Keller (988 litre ha^{-1}) had more ethanol production capacity than cv. Rio (477 litre ha^{-1}). Geren *et al.* (2011) informed that ethanol yields of cv. Keller grown as second crop under Bornova ecology were varied between 720-950 litres per hectare.

In our experiment, delaying harvest stage affected positively all tested characteristics. Except fresh biomass yield, there were significant differences between sweet sorghum cultivars in terms of other characteristics.

CONCLUSION

In conclusion, suitable harvesting time of sweet sorghum is doughy stage for high fresh biomass and ethanol yield under the ecological conditions of Bornova, Turkey. Sweet sorghum cultivar "Keller" can be preferred because of the high yield potential for growing as summer second crop.

REFERENCES

Almodares, A., Taheri, R., Hadi, M. R., Fathi, M. (2006): The effect of nitrogen and potassium fertilizers on the growth parameters and the yield components of two sweet sorghum cultivars, Pakistan Journal of Biological Sciences, 12(9):2350-2353.

Almodares, A., Hadi, M. R., Ranjbar, M., Taheri, R. (2007): The effects of nitrogen treatments, cultivars and harvest stages on stalk yield and sugar content in sweet sorghum, Asian Journal of Plant Sciences, 6(2):423-426.

Almodares, A., Taheri, R., Adeli, S. (2008): Stalk yield and carbohydrate composition of sweet sorghum (*Sorghum bicolor* (L.) Moench) cultivars and lines at different growth stages, Malaysian Applied Biology, 37(1):31-36.

Almodares, A., Ranjbar, M., Hadi, M. R. (2010): Effects of nitrogen treatments and harvesting stages on the aconitic acid, invert sugar and fiber in sweet sorghum cultivars, Journal of Environmental Biology, 31(6):1001-1005.

Anonym (2010): Technical instruction for measuring agricultural values, T.R. Ministry of Agriculture and Rural Affair, The Center of Seed Registration and Certification, Sorghum (*Sorghum spp*.), 13p. Ankara, Turkey.

Bhairappanavar, S. T., Sharanappa, A. (2011): Bio-ethanol production potential of sweet sorghum *(Sorghum bicolor)* cultivars under different spacing and harvest stages. Indian Journal of Agronomy, 56(1):52-56.

Borade, R. M., Dalvi, U. S., Lokhande, P. K., Naik, R. M., Shinde, M. S. (2015): Influence of stage of harvesting on composition of sweet sorghum, A Quarterly Journal of Life Sciences, 12(1B):219-223.

Chavan, U. D., Patil, J. V., Shinde, M. S. (2009): An assessment of sweet sorghum cultivars for ethanol production, Sugar Technology, 11(4):319-323pp.

Clegg, M. D., Gorz, H. J., Maranville, J. W., Haskins, F. A. (1986): Evaluation of agronomic and energy traits of Wray sweet sorghum and the N39 x Wray hybrid, Energy in Agriculture, 5:49-54.

Geren, H., Avcıoğlu, R., Kavut, Y. T., Sakinoğlu Oruç, Ç., Öztarhan, H. (2011): A preliminary study on the yield and some yield related characteristics of second crop sweet sorghum (*Sorghum bicolor* (L.) Moench var. *saccharatum*), National 4th Seed Congress, 14-17 June 2011, Turkey, 2:525-530.

Gibbons, W. R., Westby, C. A., Dobbs, T. L. (1986): Intermediate-scale, semicontinuous solid-phase fermentation process for production of fuel ethanol from sweet sorghum, Applied and Environmental Microbiology, 51(1):115-122.

Hugar, A. Y., Jayadeva, H. M., Rangaswamy, B. R., Shivanna, S., Chandrappa, H. (2010): Assessing the effect of nitrogen and harvesting stages on yield and yield attributes of sweet sorghum genotypes, Agricultural Science Digest, 30(2):139-141.

Lizarazu, W. Z., Monti, A. (2015): An integrated approach to harvest and storage of sweet sorghum at farm scale, Bioenergy Research, 8:450-458.

Oyier, M. O., Owuoche, J. O., Oyoo, M. E., Cheruiyot, E., Mulianga, B., Rono, J. (2017): Effect of harvesting stage on sweet sorghum (*Sorghum bicolor* L.) genotypes in Western Kenya, Hindawi, The Scientific World Journal, doi.org/10.1155/2017/8249532.

Ramesha, Y. M., Sharanappa, A. (2013): Effect of harvesting stages on juice quality and ethanol yield in sweet sorghum (*Sorghum bicolor*) cultivars during rabi, Indian Journal of Agronomy, 58(3):368-371.

Reddy, B. V. S., Ramesh, S., Reddy, P. S., Ramaiah, B., Salimath, P. M., Kachapur, R. (2005): Sweet sorghum- A potential alternate raw material for bio-ethanol and bioenergy, International Sorghum and Millets Newsletter, 46: 79–86.

Reddy, B. V. S., Sanjana, R. P. (2003): Sweet sorghum: characteristics and potential. International Sorghum and Millets Newsletter, 44:26–28.

Ricaud, R., Arceneaux, A. (1990): Sweet sorghum research on biomass and sugar production in 1990, Report of projects, Louisiana Agricultural Experiment Station, Department of Agronomy, Louisiana State University, 136-139.

Ryan, J. G., Spencer, D. C. (2001): Future challenges and opportunities for agricultural R&D in the semi-arid tropics. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 83.

SAS Institute (1998): INC SAS/STAT user's guide release 7.0, Cary, NC, USA.

Stell, R. G. D., Torrie, J. A., Dickey, D. A. (1997): Principles and Procedures of Statistics, A Biometrical Approach 3rd Edi. Mc Graw Hill Book. INC. NY.

Tsuchihashi, N., Goto, Y. (2004): Cultivation of sweet sorghum (*Sorghum bicolor* (L.) Moench) and determination of its harvest time to make use as the raw material for fermentation, practiced during rainy season in dry land of Indonesia, Plant Production Science, 7(4):442-448.

Xuan, T. D., Phuong, N. T., Khang, D. T., Khan, T. D. (2015): Influence of sowing times, densities, and soils to biomass and ethanol yield of sweet sorghum, Sustainability, 7:11657-11678.

Zhao, Y. L., Dolat, A., Steinberger, Y., Wanga, X., Osman, A., Xie, G. H. (2009): Biomass yield and changes in chemical composition of sweet sorghum cultivars grown for biofuel, Field Crops Research, 111:55–64.

EFFECT OF DIFFERENT HARVEST STAGES ON SOME SILAGE QUALITY CHARACTERISTICS OF SWEET SORGHUM (*Sorghum bicolor var. saccharatum*) AND BEAN (*Phaseolus vulgaris*) MIXTURES *

Hakan Geren[1], Gözde Durul[1]

Original scientific paper

Summary

Sweet sorghum is ensiled to preserve its nutritive value and it has high metabolic energy value, but crude protein is low. This study was conducted to assess silage and forage values of sweet sorghum (*Sorghum bicolor var. saccharatum*) silage when ensiled with different proportions of bean [*Phaseolus vulgaris*]. Rio cv. of sweet sorghum and Noyanbey-98 cv. of bean were used as crop material. Sweet sorghums were cut 3 different harvesting stages (panicle emergence, anthesis and doughy) and were mixed with bean at sorghum crop:bean ratios of 100:0%, 75:25%, 50:50%, 25:75%, and 0:100%. All crops were chopped using a conventional chopper, and for each mixture 500 g of fresh material was vacuum sealed in a plastic bag and fermented for an average of 40 days, four bags per mixture. Some yield and quality parameters were tested in the experiment such as dry matter (DM) yield, content of lactic & acetic acids, pH of silage, metabolisable energy (ME), NDF and ADF content. There were significant differences between harvest stages and mixture rates. Delaying harvest stage affected positively on DM yield and fermentation quality but not ME, NDF or ADF. Crude protein (CP) increased as proportion of bean increased in the mixture. In addition, pH and AA increased when bean was added. Silage with 100% bean without sweet sorghum had the highest pH and AA concentration.

Keywords: *Sweet sorghum, bean, harvest stage, silage proportion, silage quality.*

INTRODUCTION

In most ruminant production systems, livestock derive between 40 and 90% of their feed requirements from forages. Haymaking and ensiling are the only options available to farmers wanting to conserve forage on a large scale. Ensiling offers many advantages over haymaking: large quantities of forage can be conserved in a short time, forage conservation is less weather dependent and silage is well suited to mechanization (Charmley, 2001).

---

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] University of Ege, Faculty of Agriculture, Department of Field Crops, Izmir-Turkey
Correspondence: Hakan Geren, hakan.geren@ege.edu.tr

Corn (*Zea mays*) silage is a major forage source for dairy cows in Turkey because of its relatively constant nutritive value, high yield, and high net energy for lactation requirements ranging from 1170 Kcal/kg to 1210 Kcal/kg dry matter (DM) compared with other forage crops. However, its crude protein (CP) concentration is low, ranging from 5.5 to 7.0% DM (Qu *et al*., 2013), and, corn forage yield for silage and its quality are influenced by many interacting environmental, cultural, production costs (hybrid seed, irrigation, etc.) and genetic factors. Therefore, the producers are trying to reduce production costs by better use of grazing and alternative crops for silage making. Sweet sorghum (*Sorghum bicolor* (L.), Moench *var. saccharatum*) may be one of those alternatives to produce large amount of high quality roughage (feed) instead of corn.

Sweet sorghum belongs to a group of sorghum (*Sorghum bicolor*), and, it is known to be an annual C4 plant of tropical origin and is an interesting crop. High concentrations of its sugar juice can be extracted from the stem by milling. It is well adapted to sub-tropical and temperate regions, being highly biomass productive and water efficient. Sweet sorghum, which can be grown under widely differing climatic conditions, has been identified as a promising crop with the potential to provide for a wide spectrum of food, forage or energy uses. Sweet sorghum is ensiled to preserve its nutritive value and it has high metabolisable energy value. However, the CP content of sweet sorghum silage is also low like corn, so supplementation with protein feeds is needed to fulfill the protein requirements for producing ruminants (Stoltz *et al*., 2013).

Protein is the first critical nutrient needed to meet animal requirements when feeding sorghum silage. Untreated sweet sorghum silage has from 7 to 8% protein on a dry matter basis, and the protein requirements of beef cattle and sheep range from 10 to 14%, depending on the stage of production and desired rate of gain. High production dairy cows have even higher requirements. One way to increase the protein content in sweet sorghum forage is by intercropping sorghum with legumes (Stoltz *et al*., 2013). Many studies have confirmed the advantages of intercropping compared with monocropping, which include higher total yield per unit land area and have been conducted to assess the benefits to nutritive value of combining sorghums with annual legumes (Contreras-Govea *et al*., 2013).

Legumes have long been recognized as a good source of CP. Intercropping sorghum with legumes is a viable option to increase forage protein content and thereby improve forage quality through the synergistic effects of two or more crops grown simultaneously. Intercropping sorghum with legumes can also enhance the fermentation characteristics, CP concentration, and overall nutritive value of silages (Qu *et al*., 2013). Similarly, Armstrong *et al*. (2008) found that lablab bean intercropped with corn had the greatest potential among the climbing beans to increase CP concentration compared with monoculture corn.

The bean (*Phaseolus vulgaris*) is a common legume cultivated for its edible seeds all over the world. It is slightly hairy with a well-developed root system and the stems are many branched. The bean is a fast growing, warm season legume, and, it can grow in a diverse range of environmental conditions worldwide because of its adaptability. There are many varieties of beans grown in all the regions. However, selecting high yielding (seed and herbage), disease resistant variety is most important factor for successful cultivation. In addition, the bean serves as an adequate source of protein. Furthermore, it can be planted alone or intercropped with other crops such as corn and sorghums.

It is clear that mixing legume crops with sorghum silage should increase CP concentration, but it would also increase other constituents, such as NDF, lactic acid, and total acids concentrations and would potentially decrease digestibility (Contreras-Govea *et al.*, 2011). Therefore, it is important to determine the proper combination of legume crop that will result in the optimum mixture for nutritive value and fermentation. In addition, little research has been conducted on sweet sorghum for silage when in mixture with a legume crop. Ensiling sweet sorghum with bean provide a feasible option to increase CP concentration in silage. The objective of the study was to evaluate the nutritive value and fermentation profile of sweet sorghum silage when mixed with different proportions of bean.

MATERIALS AND METHODS

The experiment was carried out second crops production season in 2013 at Bornova experimental fields and laboratory of Agricultural Faculty of Ege University, Izmir, Turkey, at about 20 m above sea level with typical Mediterranean climate characteristics. Seeds of sweet sorghum cultivar ‘Rio’ and bean cultivar ‘Noyanbey-98’ were sown on 1 July 2013, in separate fields at a plant density of 57,143 (70 cm x 25 cm) and 285,714 (70 cm x 5 cm) plants per hectare, respectively.

Based on soil test results, sweet sorghum was fertilized with 220 kg N and 80 kg P_2O_5 per hectare; bean was fertilized with 25 kg N and 120 kg P_2O_5 per hectare. Half a dose of N fertilizer (urea) and full dose of P (triple superphosphate) were applied before planting, and the rest of nitrogen was applied when the sweet sorghum crops were 50-60 cm plant height as NH_4NO_3. Drip irrigation system was installed on the field during the establishment and growing seasons. Weed control was performed by manual hoeing only. No evident crop diseases or insects were detected.

Sweet sorghums were cut 3 different harvesting stages (panicle emergence, anthesis and doughy) and bean which was harvested a day before, was at vegetative stage with some legumes. Fresh yields of the crops were recorded. Bean was wilted for 24 h before chopping. Sweet sorghum and wilted bean were chopped separately using a static precision-chop forage harvester to give a chop length of 5–10 mm. After

chopping, approximately 15 kg of fresh material were collected in separate plastic bags for each crop and taken to the laboratory to make mixtures. Five sweet sorghum:bean mixtures (on wet weight basis) were hand-made using: (*i*) 100:0, (*ii*) 75:25, (*iii*) 50:50, (*iv*) 25:75, and (*v*) 0:100 ratios, respectively. Samples of mixture (500 g) were vacuum-packed (Johnson *et al*., 2005) into polythene bags (dimensions 30-20 cm) with addition of 0.5% salt (*NaCl*). No inoculant was applied to any combination. There were four plastic bags for each combination and bag was considered the experimental unit. The vacuum bag silos were kept in storage (room temperature) without light for 40 days for anaerobic fermentation.
Dry matter (DM) content, pH value, lactic and acetic acids of matured silage samples was determined (Alcicek and Ozkan, 1996). Matured silage samples of each component were dried at 65ºC for 48 h. The dried samples were reassembled and ground in a mill passed through a 1 mm screen. Crude protein (CP), crude fiber (CF), ether extractable fat (EEF) and total ash of forage were determined using standard techniques as prescribed by AOAC (1990). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations were analysed by the sequential detergent analysis method (Van Soest *et al.*, 1991). Metabolisable energy (ME, kcal/kg) was calculated by the formula (TSE, 2004), $ME = 3260 + (0.455 \times CP_{\%}) + (3.517 \times EEF_{\%}) - (4.037 \times CF_{\%})$. All data were statistically analyzed using analysis of variance (ANOVA) with the Statistical Analysis System (SAS, 1998). Probabilities equal to or less than 0.01 were considered significant. If ANOVA indicated differences between treatment means, a LSD test was performed to separate them (Stell *et al*., 1997).

RESULTS AND DISCUSSION

Dry matter (DM) yield: The results showed that the effect of harvest stage on DM yield of sweet sorghum and bean was significant (Table 1). DM yield was highest (11.20 t ha^{-1}) at doughy stage and lowest (5.98 t ha^{-1}) at panicle emergence stage. A delay in harvesting stage after flowering increased sweet sorghum and bean DM yields. Thus, the plants did have adequate opportunity for photosynthesis and their height and stem diameter capacity increased. These results are in agreement with the results of Atis *et al*. (2012) and Zhao *et al*. (2012) on sorghum. Moreover, Cakmakci *et al*. (1999) stated that with the delay in harvest date caused an increase in DM yield.

Table 1. Yield, silage fermentation and forage quality characteristics of sweet sorghum fermented with different bean proportions under different harvest stages.

Silage proportion	PES	AS	DS	Mean	PES	AS	DS	Mean	PES	AS	DS	Mean
	Harvest stages of sweet sorghum											
	Dry matter yield (t ha^{-1}) --				Silage pH				DM content of silage (%)			
100S+0B	5.98	8.75	11.20	8.64	4.39	4.23	3.60	4.07	25.5	25.4	24.7	25.2
75S+25B	-	-	-	-	4.52	4.29	3.88	4.23	25.0	26.1	25.7	25.6
50S+50B	-	-	-	-	4.71	4.45	4.01	4.39	25.9	25.6	25.5	25.6
25S+75B	-	-	-	-	4.87	4.73	4.65	4.75	25.7	25.3	25.6	25.5
0S+100B	2.34	3.40	4.63	3.46	5.37	4.91	4.81	5.03	24.8	25.8	25.9	25.5
Mean	8.32	12.15	15.83	12.10	4.77	4.52	4.19	4.49	25.1	25.6	25.3	25.3
LSD(.01)	HS:1.16				SP:0.12 HS:0.09 SPxHS:0.21				SP:ns HS:ns SPxHS:ns			
	Lactic acid content (%)				Acetic acid content (%)				Crude protein content (%)			
100S+0B	1.09	2.38	2.79	2.09	0.21	0.14	0.09	0.15	8.01	7.78	6.86	7.55
75S+25B	0.82	2.06	2.65	1.84	0.33	0.23	0.16	0.24	9.02	8.19	8.03	8.42
50S+50B	0.80	1.50	2.31	1.54	0.52	0.33	0.30	0.38	13.13	11.63	11.26	12.01
25S+75B	0.72	1.20	1.77	1.23	0.66	0.54	0.37	0.52	16.25	15.66	14.47	15.46
0S+100B	0.68	0.93	1.07	0.89	0.83	0.62	0.42	0.63	23.10	20.36	18.55	20.67
Mean	0.82	1.62	2.12	1.52	0.51	0.37	0.27	0.38	13.90	12.73	11.83	12.82
LSD(.01)	SP:0.13 HS:0.09 SPxBD:0.22				SP:0.05 HS:0.04 SPxHS:0.08				SP:0.61 HS:0.47 SPxHS:1.05			
	NDF content (%)				ADF content (%)				Metabolisable energy (kcal kg^{-1})			
100S+0B	45.4	48.3	50.2	48.0	35.3	40.0	42.0	39.1	2253	2141	2072	2155
75S+25B	45.0	46.5	49.2	46.9	34.1	38.6	40.6	37.8	2270	2175	2100	2182
50S+50B	43.3	45.4	48.5	45.8	33.3	37.9	39.9	37.0	2331	2222	2114	2222
25S+75B	41.3	45.0	47.7	44.6	33.3	36.5	38.5	36.1	2390	2292	2209	2297
0S+100B	40.5	44.0	46.2	43.5	32.7	35.3	37.3	35.1	2528	2452	2280	2420
Mean	43.1	45.8	48.3	45.8	33.7	37.7	39.7	37.0	2354	2257	2155	2255
LSD(.01)	SP:1.2 HS:0.9 SPxHS:ns				SP:1.0 HS:0.8 SPxHS:1.8				SP:61 HS:47 SPxHS:ns			

PES: panicle emergence stage, AS: anthesis stage, DS: doughy stage,
HS: harvest stages, SP: silage proportions, ns: not significant.

Silage pH: There was significant interaction between harvest stages and silage proportions in terms of silage pH (Table 1). The lowest pH of silage obtained from sweet sorghum (3.60) with 0% bean, which is an indicator of good fermentation cut at doughy stage. In contrast, silage with 100% bean cut at panicle emergence stage had the highest (undesirable) pH (5.37), which is probably an indicator of clostridia fermentation (McDonald *et al.*, 1991). This result shows that pH values increased with addition of bean to the mixtures and decrease by delaying of harvest stages. The most important physicochemical parameter for the evaluation of silage quality is a pH value 4.2 or below, which was observed for sweet sorghum silage from 0% to 50% bean was added (McDonald *et al.*, 1991; Kung and Shaver, 2001). As it known that total sugar in juice of sweet sorghum is relatively high compared to other annual crops for making silage like corn or sorghum hybrids (Almodares *et al.*, 2007; Kumar *et al.*, 2008). The juice sugar content depended on the plant stage of development, because at the early development stage, fructose is more abundant, whereas sucrose is dominant after heading (Almodares *et al*., 2006). At doughy stage, the sweet sorghum juice sugar content (brix) was average 22% (not seen the table) in our experiment. Sugars are the substrates for the fermentation process, so that their concentration in the crop has a major influence on the extent and type of fermentation in silage. It appears that high sugar concentration in fresh sweet sorghum gives a high probability of lactate type silage and of the silage being well preserved. In the study, bean increased in the mixture from 50% to 100% increased the pH value from 4.39 to 5.03 and the fermentation type was acetate.

DM content of silage: Harvest stages or silage proportion had no significant effects on the DM content of silage in our experiment (Table 1). DM content of silage ranged from 24.7% to 26.1% depending on the harvest stages and silage proportion. DM concentration was in the range recommended for ensiling in sorghum crop by wilting procedures. Many researchers (Comberg, 1974; Woolford, 1984; McDonald *et al*., 1991) informed that wilting is recognized to be an effective procedure in silage making but wet forage, a low buffering capacity like legume crops, was difficult to ensile, because high moisture content generally promotes the development of clostridial fermentation and also dilutes plant sugar concentrations and slows the decline in silage pH (Yunus *et al*., 2000).

Lactic (LA) and Acetic (AA) acid contents: There were significant interaction between harvest stages and silage proportions in terms of LA and AA concentrations of silage (Table 1). The highest LA (2.79%) concentrations was obtained at pure sweet sorghum silage cut at doughy stage while the lowest LA content of silage (0.68%) was obtained pure bean silage cut at the first maturity stage. On the other hand, pure sweet sorghum silage cut at doughy stage had the lowest AA concentration (0.09%), whereas pure bean silage cut at the first maturity stage was the highest (0.83%). In our present experiment, LA concentration decreased linearly as increasing level of bean addition in the mixture and increased by delaying harvest stages. Vice versa, AA concentration also decreased as decreasing level of bean addition to the silage and delaying cutting stages. Similar results were obtained by Contreras-Govea *et al*. (2011 and 2013) and Armstrong *et al*. (2008). It appears that addition of bean (below 50%) in to sweet sorghum silage had acceptable level under anthesis and doughy stage; however, higher rate of addition bean material had negative effect on silage fermentation properties.

Crude protein (CP) content: Significant interaction was detected on protein content of silage samples in the experiment. The highest CP content (23.10%) was recorded at pure bean silage cut at the first maturity stage as compared to pure sweet sorghum silage (minimum: 6.88%) cut at the third maturity stage in our experiment. Generally, average CP concentration increased linearly from 7.55% to 20.67% as the amount of bean increased from 0 to 100% in the mixture, and decreased from 13.90% to 11.83% by delaying harvest stage. This response is in agreement with previous research (Geren *et al*., 2008; Contreras-Govea *et al*., 2011-2013). Sweet sorghum is by nature low in crude protein (Geren *et al*., 2013). Therefore, adding a legume such as bean or soybean, which is high in CP, would be expected to increase CP in the mixture. Previous field intercropping studies (Carruthers *et al*., 2000; Armstrong *et al*., 2008; Kavut *et al*., 2015) had reported that planting corn and legume at the same time increased CP concentration from 12.9 to 29.0%. Contreras-Govea *et al*. (2011) ensiled corn and forage sorghum with different proportions of lablab bean and reported that legume must make up at least 50% of the mixture to affect fermentation and nutritive value. They found that quality traits such as pH, AA, CP, NDF or ADF concentration were higher as the proportion of lablab bean in the mixture increased. Similar results were reported by Geren *et al*. (2008) and Contreras-Govea *et al*. (2013), who assessed the fermentation of corn silage in mixture with cowpea or other climbing beans. They also reported that, compared to corn alone, CP concentration increased in the mixture, but pH, NDF, and lactic acid concentration also increased, with no significant difference in in-vitro digestibility.

NDF and ADF contents: Harvest stage and silage proportion had significant effects on the NDF and ADF content of silage in the experiment, and for ADF, interaction was also significant. The highest average NDF content was 48.3% for the latest harvesting time, while the lowest NDF content was 43.1% for the first harvesting time. Average NDF content decreased from 48.0% to 43.5% as bean increased in the mixture from 0% to 100%. On the other side, the lowest ADF content (32.7%) was recorded in bean silage without sweet sorghum in the first cutting time, whereas the highest ADF content was 42.0% for pure sweet sorghum silage cut at the latest harvest stage in our experiment. Generally, ADF content decreased linearly in sweet sorghum silage as bean proportion increased in the mixture. These results are in disagreement with those reported by Contreras-Govea *et al*. (2011-2013), evaluating grass (corn or sorghum)–legume (cowpea or lablab bean) mixtures, and by Lima *et al*. (2010), assessing sorghum–soybean mixture, in which NDF or ADF concentration increased with the addition of legume to corn or sorghum. As expected, cell wall concentrations (NDF, ADF, etc.) were the lowest in the immature plants (panicle emergence stage) and gradually increased as the plants matured (doughy stage). Many researchers stated that the nutritional value of cell wall components decreased with plant age was related to increased lignin content (Atis *et al*. 2012; Zhao *et al*., 2012).

Metabolisable energy (ME): There were statistically significant differences among harvest stages and silage proportions regarding average metabolisable energy levels. The highest average ME was 2354 kcal/kg for first harvesting stage, while the lowest ME was 2155 kcal/kg for the third harvesting stage. On the other side, the highest average ME was obtained from pure bean silage (2420 kcal/kg), whereas the lowest average ME obtained from pure sweet sorghum silage being 2155 kcal/kg, but there was no significant difference between 0%, 25% or 50% bean addition. ME values increased as the amount of bean increased from 0 to 100% in the mixture. Contreras-Govea *et al*. (2013) ensiled corn and forage sorghum with different proportions of cowpea, reported net energy of lactation was significantly affected by crop and cowpea addition to the mixtures. They also found that energy values were higher as the proportion of cowpea in the mixture decreased. Previous studies (Geren *et al*., 2008; Geren, 2014) about improving of ME of grass silage displayed that some additives to the crop at the time of ensiling process had greater effect on ME.

CONCLUSIONS

It was concluded that bean addition to sweet sorghum silage had significant effect on silage quality but harvest stages of sorghum had greater effect on the forage DM yield. Addition of bean compensates for the low crude protein content of sweet sorghum and also affecting other nutritive value characteristics in silage. Soft doughy stage can be recommended for suitable harvest stage of sweet sorghum for silage production. However, a greater benefit in nutritive value was observed when bean was between 25 and 50% of the mixture. Additional research activities with rumen digestibility are needed to assess mixtures that produce silage with more desirable fermentation characteristics.

REFERENCES

Alcicek, A. and Ozkan, K. (1996): Zur quantitativen Bestimmung von Milch-, Essig- und Buttersäuren in Silage mit Hilfe eines Destillationsverfahren, J.of Agr.Fac.of Ege Univ, 33(2-3):191-198.

Almodares, A., Hadi, M. R., Ranjbar, M., Taheri, R. (2007): The effects of nitrogen treatments, cultivars and harvest stages on stalk yield and sugar content in sweet sorghum, Asian Journal of Plant Sciences, 6(2), 423-426.

Almodares, A., Taheri, R., Hadi, M. R., Fathi, M. (2006): The effect of nitrogen and potassium fertilizers on the growth parameters and the yield components of two sweet sorghum cultivars, Pakistan Journal of Biological Sciences, 12(9), 2350-2353.

AOAC (1990): Official methods of analysis, 15th ed. Association of Official Analytical Chemists, Arlington, Virginia, pp. 84-85.

Armstrong, K. L., Albrecht, K. A., Lauer, J. G., Riday. H. (2008): Intercropping corn with lablab bean, velvet bean, and scarlet runner bean for forage, Crop Sci., 48:371–379.

Atis, I., Konuskan, O., Duru, M., Gozubenli, H., Yilmaz, S. (2012): Effect of harvesting time on yield, composition and forage quality of some forage sorghum cultivars. Int. J. Agric. Biol., 14:879-886.

Cakmakci, S., Gunduz, I., Cecen, S., Aydinoglu, B. (1999): Effects of different harvesting times on yield and quality of sorghum silage (*Sorghum bicolor* L.), Turkish J. Agric. For., 23:603–611.

Charmley, E. (2001): Towards improved silage quality – A review, Can. J. Anim. Sci. 81:157–168.

Comberg, G. (1974): Gärfutter: Betriebswirtschaft, Erzeugung, Verfütterung, Verlag Eugen Ulmer Stuttgart, Gerokstraße 19, Printed in Germany, ISBN:3-8001-4321-6, 260s.

Contreras-Govea, F. E., VanLeeuwen, D. M., Angadi, S. V., Marsalis, M. A. (2013): Enhances in crude protein and effects on fermentation profile of corn and forage sorghum silage with addition of cowpea, Forage and Grazinglands. doi:10.1094/FG-2013- 0622-01-RS.

Contreras-Govea, F., Marsalis, M., Angadi, S., Smith, G., Lauriault, L. M., VanLeeuwen, D. (2011): Fermentability and nutritive value of corn and forage sorghum silage when in mixture with lablab bean, Crop Sci. 51:1307–1313.

Geren, H., Avcioglu, R., Soya, H., Kır, B. (2008): Intercropping of corn with cowpea and bean: Biomass yield and silage quality, African Journal of Biotechnology, 7(22):4100-4104.

Geren, H. (2014): An investigation on some quality characteristics of ensilaged giant kinggrass (*Pennisetum hybridum*) with different levels of leguminous forages, The Journal of Ege University, Faculty of Agriculture, 51(2): 209-217.

Geren, H., Avcioglu, R., Girgin, V. Ç. (2013): Effects of different nitrogen levels on stalk yield and ethanol productivity of sweet sorghum (*Sorghum bicolor* (L.) Moench *var. saccharatum*) under Mediterranean climatic conditions, Proceedings of 24th International Scientific-Expert Conference of Agriculture and Food Industry, Sarajevo, September 25 – 28, 2013, 323-325.

Johnson, H. E., Merry, R. J., Davies, D. R., Kell, D. B., Theodorou, M. K., Griffith, G. W. (2005): Vacuum packing: a model system for laboratory-scale silage fermentations, Journal of Applied Microbiology, 98:106–113.

Kavut, Y. T., Geren, H., Avcioglu, R., Soya, H. (2015): Effects of previous legume crop levels of nitrogen and sowing date on yield components and some morphological characteristics of corn, Legume Research, 38(3):341-347.

Kumar, S. R., Shrotria, P. K., Deshmukh, J. P. (2008): Characterizing of nutrient management effect on yield of sweet sorghum genotypes, World Journal of Agricultural Science, 4, 787-789.

Kung, L., Shaver, R. (2001): Interpretation and use of silage fermentation analysis reports, Focus on Forage. 3(13):1-5. Univ. of Wisconsin Ext., Madison, WI.

Lima, R., Lourenco, M., Diaz, R. F., Castro, A., Fievez, V. (2010): Effect of combined ensiling of sorghum and soybean with and without molasses and lactobacilli on silage quality and in vitro rumen fermentation, Anim. Feed Sci. Technol. 155:122–131.

McDonald, P., Henderson, A. R., Heron, S. J. E. (1991): The Biochemistry of Silage, 2nd Edition, Chalcombe Publications, Printed in Great Britain by Cambrian Printers Ltd, Aberystwyth, ISBN:0-948617-22-5, 327.

SAS Institute (1998): INC SAS/STAT user's guide release 7.0, Cary, NC, USA.

Stell, R. G. D., Torrie, J. A., Dickey, D. A. (1997): Principles and Procedures of Statistics, A Biometrical Approach 3rd Edi. Mc Graw Hill Book. INC. NY.

Stoltz, E., Nadeau, E., Wallenhammar, A. C. (2013): Intercropping maize and faba bean for silage under Swedish climate conditions, Agricultural Research, 2(1):90-97.

TSE (2004): Chemical methods of metabolisable energy for animal feed, Institute of Turkish Standards, Pub. No:9610, Ankara.

Van Soest, P. J., Robertson, J. B., Lewis, B. A. (1991): Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition, J. Dairy Sci. 74:3583-3597.

Woolford, M. K. (1984): The Silage Ferment, Grassland Research Institute, Hurley, England, 350.

Qu, Y., Jiang, W., Yin, G., Wei, C., Bao, J. (2013): Effects of feeding corn-lablab bean mixture silages on nutrient apparent digestibility and performance of dairy cows, Asian-Australian J. Anim. Sci. 26(4):509-516.

Yunus, M., Ohba, N., Shimojo, M., Furuse, M., Masuda, Y. (2000): Effects of adding urea and molasses on Napiergrass silage quality, Asian-Aus. J. Anim. Sci. 13(11):1542-1547.

Zhao, Y. L., Steinberger, Y., Shi, M., Han, L. P., Xie, G. H. (2012): Changes in stem composition and harvested produce of sweet sorghum during the period from maturity to a sequence of delayed harvest dates, Biomass Bioenerg., 39: 261–273.

GRAIN YIELD AND SOME YIELD COMPONENTS OF SWEET SORGHUM (*Sorghum bicolor var. saccharatum*) AS AFFECTED BY PLANT DENSITIES UNDER MEDITERRANEAN CLIMATIC CONDITIONS*

Hakan Geren[1], Gülcan Demiroğlu Topçu[1], Yaşar Tuncer Kavut[1], Şükrü Sezgi Özkan[1]

Original scientific paper

Summary

This study was conducted to evaluate the effects of plant density on the grain yield and some yield characteristics of sweet sorghum (*Sorghum bicolor var. saccharatum*). Sorghum was grown in summer as a second crop under Mediterranean ecological conditions of Izmir, Turkey during 2013-2014. The experiment was carried out with a randomized complete block design with three replication; five plant spacing 70 cm among the rows and 25, 20, 15, 10 and 5 cm within the rows (D_1:57,142; D_2:71,428; D_3:95,238; D_4:142,857 and D_5:285,714 plant ha^{-1}, respectively) were tested. 'Keller' cultivar of sweet sorghum was used as a crop material. Some traits were tested in the experiment such as plant height, panicle length and diameter, 1000-grain weight, harvest index, grain yield and grain CP content. Average result of two years indicated that there were significant effect of plant densities on the grain yield and some yield parameters of sweet sorghum. Some yield components like panicle length, panicle diameter and panicle weight per plant decreased as the number of plants in unit area increased (from D_1 to D_5). Densely populated stands (D_4 and D_5) had higher grain yield compared to sparsely populated stands (D_1 and D_2). However, D_3 was the most successful planting density of sweet sorghum regarding grain yield to the regions with Mediterranean-type climates under irrigation, and it is recommended for production.

Key words: *sweet sorghum, plant density, grain yield, harvest index*.

INTRODUCTION

Sorghum, a warm season tropical grass, is reported to be the most widely adapted species among cereal grasses that perform favorably in dry environments (Rutto *et al*., 2013; Zand and Shakiba, 2013). Under low soil moisture conditions, sorghum maintains its physiological activity close to that of plants with sufficient moisture by increasing root length, density, and water-use efficiency (Lafarge *et al*., 2002). Compared to other sorghums, sweet sorghum produces less grain but contains a large

---

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] University of Ege, Faculty of Agriculture, Department of Field Crops, Izmir-Turkey
Correspondence: Hakan Geren, hakan.geren@ege.edu.tr

amount of readily fermentable sugars in the stem (Mahmoud *et al.*, 1999; Moosavi *et al.*, 2012).

Sweet sorghum stem juice can be used for sugar, syrup, and ethanol production. The bagasse is also used as forage or as raw material for the paper industry (Reddy *et al.*, 2005). Because of its efficient conversion of atmospheric CO_2 into sugar, sweet sorghum is a promising crop for use in the bioenergy industry. Several characteristics make sweet sorghum suitable for bioenergy: (*i*) a short growth cycle (~4 months) that may allow for double cropping; (*ii*) easy propagation from seed; (*iii*) potential for fully mechanized production; (*iv*) dual purpose cropping for both stem sugar and grain starch; (*v*) high water and nutrient use efficiency; (*vi*) by-product (bagasse and forage) utilization for energy production; (*vii*) wide adaptability to different environments (Reddy *et al.*, 2005).

Plant density is one of the important factor determines growth, development and yield. Plant density selection for expression of maximum grain yield is a management practice that would make sorghum production more economical. Cultivation of plants with desirable density has positive effect on crop yield components, so that the suitable will be achieved by optimum plant density (El-Hattab *et al.*, 1980). However, maintenance of optimum planting density is always a big problem to the farmers. Substandard plant density result in high weeds infestation, poor radiation use efficiency and low yield, while dense plant population on the other hand cause lodging, poor light penetration in the canopy, reduce photosynthesis production due to shading of lower leaves and drastically reduce the yield (Zand and Shakiba, 2013).

The response of sweet sorghum to plant density has been investigated in many areas of the world. Investigation of growth and yield performance of sweet sorghum with special reference to arrangement has been conducted and the result showed that leaf area index, crop growth rate, stem and grain yield have increased by increasing plant density (Krishnareddy *et al.*, 2006). Mahmoud *et al.* (1999) used 50 and 70 cm inter-row spacing and 1, 2 and 3 plants hill^{-1}, they found that 70 cm row spacing produced the highest stalk diameter, brix, sucrose and stalk weight.

Mahmoud *et al.* (2013) and Moosavi *et al.* (2012) reported that, there are two general concepts to describe the relationship between row spacing, plant densities and yield. Firstly, maximum yield can be obtained only if the plant community produces enough leaf area to provide maximum isolation interception during reproductive growth. Secondly, equidistant spacing between plants will maximize yield because it minimizes inter plant competition. Mahmoud *et al.* (2013) observed that stalk yield of sweet sorghum was significantly affected by row spacing. The stalk yield of 20 cm inter-row spacing (166,666 plant ha^{-1}) was significantly higher than that of 35 cm inter row spacing (95,240 plant ha^{-1}). Turgut *et al.* (2005) indicated that yields in sweet sorghum decreased with increasing intra-row spacing, and grain yield of sweet sorghum increased with increasing intra-row spacing up to 15 cm spacing. However, no statistically significant differences were detected between 10, 15 and 20 cm intra-row spacings, with an average of 4.16 t ha^{-1} grain yield. The objective of this study was to determine to impact of different planting densities on the grain yield and some yield components of sweet sorghum under irrigated conditions of Mediterranean climate, Turkey.

MATERIALS AND METHODS

The field experiment was carried out for two growing seasons (2013-2014) at Bornova experimental fields of Agricultural Faculty of Ege University, Izmir, Turkey, at about 20 m above sea level with typical Mediterranean climate characteristics (Table 1). Some physical and chemical characteristics of experimental soil presented in Table 2.

Table 1: Some meteorological characteristics of experimental area in Bornova in 2013 and 2014

	Temperature (°C)			Total precipitation (mm)		
Months	2013	2014	LYA	2013	2014	LYA
Jan.	9.4	9.9	8.1	252.5	133.8	109.7
Feb.	11.2	9.7	8.6	187.0	45.6	89.8
March	14.0	11.5	10.8	56.8	108.4	72.3
April	17.3	15.0	15.0	30.2	76.8	48.9
May	22.7	19.3	20.2	43.7	2.2	32.2
June	25.7	23.8	25.0	27.1	75.2	8.2
July	28.4	26.8	27.6	0.0	16.0	3.6
August	28.7	28.3	27.0	20.2	6.0	2.1
Sept.	24.0	23.0	22.2	5.1	18.6	17.0
Oct.	17.2	18.8	18.0	94.1	49.1	46.8
Nov.	15.0	13.2	13.2	128.9	15.2	80.3
Dec.	8.5	11.1	9.9	9.1	206.8	122.3
$\bar{x}$ - Σ	18.5	17.5	17.1	854.7	753.6	633.2

LYA: Long year average, $\bar{x}$: mean, Σ:total

Table 2: Some physical and chemical characteristics of experimental soil

Characteristics	0-20 cm	20-40 cm
Sand (%)	24.72	32.72
Clay (%)	32.56	30.56
Silt (%)	42.72	36.72
Texture	Clay loam	Loam
pH	8.2	7.8
$CaCO_3$ (%)	21.52	18.64
Total salt (%)	0.095	0.075

Organic mat. (%)	1.132	1.151
Total N (%)	0.101	0.123
Available P (ppm)	0.39	0.41
Available K (ppm)	395	297

The experiments were carried out with a randomised complete block design with three replicates. In both years, seeds of sweet sorghum cultivar 'Keller' were used as crop material. Five plant densities [D_1:57,142; D_2:71,428; D_3:95,238; D_4:142,857 and D_5:285,714 plant ha^{-1}] were used in this study. The distance between rows was 70 cm and intra-row spacings were 25, 20, 15, 10 and 5 cm, respectively. Plot size was 2.80 m x 5 m (14 m^2), with 4 rows. Seeding was done manually at the rate of 10 $kg \cdot ha^{-1}$ on July 1 and 7 in 2013 and 2014, respectively. The rows thinned by hand after emergence to the required intra-row spacings.

Based on soil test results, sweet sorghum was fertilized with 220 kg N and 80 kg P_2O_5 per hectare. Half a dose of N fertilizer (urea) and full dose of P (triple superphosphate) were applied before sowing, and the rest of nitrogen was applied when the sweet sorghum crops were 50-60 cm plant height as NH_4NO_3. Drip irrigation system was installed on the field during the establishment and growing seasons. No herbicide was used to control weeds; only hand hoe was done twice. Alphacypermethrin was applied at 6-7 leaf stage of crops to control *Ostrinia nubilalis*. No evident crop diseases were detected.

The harvest was done during the physiological maturity of the grains (moisture ~13%) in early October and in order to measure the grain yield, the crops of two inner rows per plot were harvested while considering the marginal effects of both sides. In order to determine the yield and yield components (plant height, panicle length, panicle diameter, panicle weight per plant) ten plants were randomly selected from each replication and measured individually. Average 1000-grain weight was determined by counting and weighing four 100-grain samples. Crude protein content (%) of grain was determined using the Kjeldahl method (N %) with a conversion factor of 6.25. The collected data for the two years were subjected to analysis of variance (ANOVA) and significant differences between treatment means were determined by using least significant difference (LSD) test at $p \leq 0.05$ level (Stell *et al.*, 1997) using a computer software Statistical Analysis System (SAS, 1998).

RESULTS AND DISCUSSION

Experimental area is located in the Mediterranean zone of the country with quite mild and warm winters and hot and dry summers (Table 1). Field studies were started at the beginning of July with high air temperature and satisfactory moisture levels supported by drip irrigation, therefore, stands were excellent. The results are summarized in Table 3.

Plant height: The plant height of sweet sorghum was affected by density and year. Year effect was significant and average sweet sorghum height of first year (222.9 cm) was higher than second year (213.6 cm). The highest plant height (239.8 cm) was obtained from D_5 in 2013, whereas the lowest was 193.8 cm for D_1 in 2014. Similarly, average plant height of D_5 (237.4 cm) was higher than D_1 (200.4 cm) (Table 3). In the study, average result displayed that the plant height of sweet sorghum increased noticeably by increasing plant density (from D_1 to D_5) in both years most probably due to the thick-stemmed plants developed at wider within the rows and less competition compared to narrow spacing. These results are similar to those by Krishnareddy *et al*. (2006) found that the plant height of sweet sorghum increased with increase in plant density. Kucuksemerci (2017) stated that plant height of sweet sorghum was not influenced by increasing the plant density from 7.5 to 17.5 plants m^{-2}.

Panicle length: There was significant effect on panicle length of sweet sorghum due to the plant density during the study (Table 3). Year effect was not significant. The results of two years average showed that minimum plant density (D_1) had the highest panicle length (39.6 cm) compared to maximum plant density (D_5: 20.4 cm) in the study. Average value in the study indicated that plant density practices affected panicle length of sweet sorghum significantly, and higher plant densities (D_5 or D_4) reduced panicle length. This could be related to the supportive effects of more available fertilizers to lower number of plants per unit area, which permitted building of more vigorous growth that resulted in higher panicle length. Higher plant densities resulted in inter competition increase.

Panicle diameter: There were statistically significant differences among plant densities regarding average panicle diameter per plant (Table 3). Minimum plant density being D_1 (57,142 plant ha^{-1}) had the highest average panicle diameter (9.1 cm), whereas maximum plant density being D_5 (285,714 plant ha^{-1}) was the lowest (6.1 cm). Year effect was not significant on the panicle diameter. In our study, average result indicated that the panicle diameter of sweet sorghum increased by decreasing plant density (from D_5 to D_1) in both years. Reason of panicle diameter reduction at higher densities can be linked to a reduction of assimilate allocation and more intra-plant competition. Many researchers informed that panicle diameter of sweet sorghum affected negatively by increasing the plant density (Mahmoud *et al*., 2013; Zand and Shakiba, 2013). Lafarge *et al*. (2002) recorded that plant height, tillering capacity, stem diameter and yield responses to density could be related to general concepts associated with interplant competition and resource capture.

Panicle weight per plant: The effect of plant density on single panicle weight of sweet sorghum was significant (Table 3). Year effect was also significant and average panicle weight in the first year (122.6 g $plant^{-1}$) was higher than it was in the second year (118.1 cm). The highest average panicle weight (148.8 g $plant^{-1}$) was noticed at

D_1 (57,142 plant ha^{-1}) as compared to D_5 (285,714 plant ha^{-1}) (83.2 g plant^{-1}). Means comparison showed that under the conditions of the current study, panicle weight of sweet sorghum increased noticeably by decreasing plant density (from D_1 to D_5). The panicle weight per plant is an important factor in determining the total grain yield. The growth and development of plant depends on the condition prevailing on the ground surface as well as in the rhizosphere. It is possible that the lower number of plants per unit area helps the growth of plants because of better accessibility of light, fertilizer nutrients and water, thereby increasing the accumulation of nutritive ingredients in the panicle. These results are in agreement with those recorded by Zand and Shakiba (2013).

Table 3: Effect of different plant densities on the yield and some yield components of sweet sorghum

D	2013	2014	2 yrs aver.	2013	2014	2 yrs aver.	2013	2014	2 yrs aver.	2013	2014	2 yrs aver.
	Plant height (cm)			Panicle length (cm)			Panicle diameter (cm)			Panicle weight (g plant^{-1})		
D_1	207.1	193.8	200.4	39.5	39.8	39.6	9.0	9.2	9.1	151.0	146.7	148.8
D_2	212.4	204.8	208.6	34.7	36.8	35.8	8.8	8.8	8.8	138.0	137.0	137.5
D_3	224.2	208.7	216.5	33.1	33.0	33.1	8.6	8.8	8.7	133.7	126.7	130.2
D_4	230.9	225.6	228.2	28.4	26.9	27.7	7.4	7.6	7.5	104.7	99.7	102.2
D_5	239.8	235.0	237.4	21.0	19.7	20.4	5.9	6.3	6.1	85.7	80.7	83.2
Mean	222.9	213.6	218.2	31.3	31.2	31.3	8.0	8.1	8.0	122.6	118.1	120.4
LSD	Y:4.6	D:7.2	YxD:ns	Y:ns	D:2.6	YxD:ns	Y:ns	D:0.5	YxD:ns	Y:3.1	D:4.9	YxD:ns
	1000 grain weight (g)			Harvest index (%)			Grain yield (kg ha^{-1})			Grain CP content (%)		
D_1	27.0	26.6	26.8	33.3	34.4	33.8	2163	2050	2106	10.5	10.7	10.6
D_2	25.9	25.4	25.7	30.0	30.8	30.4	2582	2435	2508	10.1	10.1	10.1
D_3	25.4	25.3	25.3	29.5	30.5	30.0	3186	3065	3125	9.0	9.2	9.1
D_4	22.2	23.1	22.7	26.8	24.9	25.9	2868	2739	2804	8.9	8.9	8.9
D_5	19.6	19.3	19.5	25.2	23.7	24.5	2622	2627	2625	8.6	8.8	8.7
Mean	24.0	24.0	24.0	29.0	28.9	28.9	2684	2583	2634	9.4	9.5	9.5
LSD	Y:ns	D:0.7	YxD:ns	Y:ns	D:1.3	YxD:ns	Y:47	D:75	YxD:ns	Y:ns	D:0.4	YxD:ns

Y: year, D: density, YxD: interaction, ns: not significant,
(D_1: 70 x 25 cm; D_2: 70 x 20 cm; D_3: 70 x 15 cm; D_4:70 x 10 cm D_5:70 x 5 cm)

1000-grain weight: Plant densities exhibited significant positive effects on thousand-grain weight. Thousand grain weight was higher (27.0 g and 26.6 g) for the lowest plant density (D_1: 57,142 plants ha^{-1}) compared to 19.6 g and 19.3 g for the highest plant density (D_5: 285,714 plants ha^{-1}) in both growing seasons, respectively. Means of data obtained in Tables 3 show that thousand-grain weight of sweet sorghum was significantly decreased with increase in plant density from 57,142 to 285,714 plants ha^{-1} in our study. These results in accordance with those reported by Mahmoud *et al.* (2013) and Mekdad and Rady (2016).

Harvest index: The ANOVA results showed that harvest index of sweet sorghum was significantly affected by the plant density at 5% probability level. The interactive effect of year and density was not significant. Our results showed that the maximum amount of harvest index (33.8%) was obtained by application of 25 cm intra-raw distance (D_1) however, the minimum amount (24.5%) obtained by application of 5 cm intra-raw spacing (D_5). There were no significant differences between D_2 and D_3. In our study, the results of two years average indicated that decreasing plant densities from D_1 to D_5 decreased harvest index. The productive capacity of any crop plant depends, not only on its photosynthetic efficiency, but also on the effective translocation of assimilates to the seeds, which is measured by the harvest index. This partitioning between vegetative and reproductive parts can be modified by agronomic practices such as sowing date, plant density, fertilization and irrigation, etc. (El-Aref, 2005; Moosavi *et al*. 2012). Our findings were in agreement with some researchers (Zand and Shakiba, 2013; Mekdad and Rady, 2016).

Grain yield: Results showed that grain yield of sweet sorghum was significantly affected by the plant density and year (Table 3). Grain yield in the crops ranged from 2050 kg to 3186 kg ha^{-1} in both growing season. The highest grain yield (3125 kg ha^{-1}) was found at D_3, whereas the lowest yield (2106 kg ha^{-1}) was at D_1. Year effect was also significant indicating better humidity conditions for the maturation of crops in 2013 compared to 2014, and, average grain yield of sweet sorghum in the first year (2684 kg ha^{-1}) was higher than the second year (2583 kg ha^{-1}). In our study, the grain yield of sweet sorghum noticeably increased by increasing plant density from D_1 to D_3 but later slightly decreased (D_3 to D_5). Although highest yield was obtained with D_3 (95,238 plant ha^{-1}) in the study, the highest values of growth components were obtained with the lowest density (D_1). This may be resulted from more space; more availability to light, mineral nutrients, and low inter plants competition (Mokadem, 1994). Concerning, the reduction in vegetative traits accompanied with higher densities may be due to lower photosynthetic rate because of greater shading effect under higher densities. In other words, although increasing yield component per plant in sparsely populated stand, lacks of number of plant per unit area in lower density did not compensate the yields. These results in accordance with those reported by Mahmoud *et al*. (2013). Mekdad and Rady (2016) informed that there was a significant difference on the grain yield among the different plant densities in sweet sorghum, and grain yield increased as plant density increased (111,000; 133,000 and 166,000 plant ha^{-1}), and higher grain yields were obtained from 166,000 plant ha^{-1} in the first (2.38 t ha^{-1}) and second (2.48 t ha^{-1}) year, respectively. Turgut *et al*. (2005) pointed out that grain yield of sweet sorghum increased with increasing intra-row spacing up to 15 cm spacing. However, no statistically significant differences were detected between 10, 15 and 20 cm intra-row spacings.

Grain crude protein (CP) content: Plant density exhibited significant effects on grain CP content of sweet sorghum and, CP content varied between 8.6% and 10.7%

in both growing season (Table 3). The effect of year was not significant. The highest CP content (10.6%) was measured at D_1 (57,142 plant ha^{-1}), whereas the lowest CP content (8.7%) was at D_5 (285,714 plant ha^{-1}). Grain CP content of sweet sorghum decreased with decreasing intra-row spacing from 25 cm to 5 cm. However, no statistically significant differences were detected among 15, 10 or 5 cm intra-row spacings (D_3, D_4, D_5). In other word, sparsely populated stands (D_1 and D_2) had higher CP content compared to densely populated stands (D_4 and D_5) during the study. Some researches (Mahmood, 2012; Cifuentes *et al*., 2014) reported that CP content of sweet sorghum did not influenced by different plant densities. Meanwhile, Afzal *et al*. (2013) informed that CP content (10.4%) of sweet sorghum was higher in densely populated stands compared to sparsely populated stands (9.7%).

In our experiment, the effect of plant density was the main source of variation in all tested characters, but not year, while the interactions (YxD) were not significant on any characteristics. Some yield components like panicle length, panicle diameter, panicle weight per plant, harvest index and grain CP contents decreased as the number of plants in unit area increased (from D_1 to D_5).

CONCLUSIONS

The results of our study testing the effect of five plant density (D_1:57,142; D_2:71,428; D_3:95,238; D_4:142,857 and D_5: 285,714 plant ha^{-1}) on the sweet sorghum showed that based on grain yield and crude protein yield, the planting of sweet sorghum using 95,238 plants ha^{-1} (70 x 15 cm) should be recommended for second crops production in the regions with Mediterranean-type climates and in similar agro-ecologies of the country.

REFERENCES

Afzal, M., Ahmad, A. U. H., Zamir, S. I., Khalid, F., Mohsin A. U. and Gillani, S. M. W. (2013): Performance of multicut forage sorghum under various sowing methods and nitrogen application rates, J Anim. Plant Sci, 23(1):232-239.

Cifuentes, R., Bressani, R. and Rolz, C. (2014): The potential of sweet sorghum as a source of ethanol and protein, Energy for Sust. Dev., 21:13–19.

El-Aref, A. O. (2005): Effect of plant density on growth, yield and quality on some sweet sorghum varieties, Alexandria. Sci. Exchange J., 26(3):247-254.

El-Hattab, H. S., Hussein, M. A., El-Hattab, A. H., Raouf M. S. A. and El-Nomany, A. A. (1980): Growth analysis of maize plant in relation to grain yield as affected by nitrogen levels, Z Acker- Pflanzenbau, 149:46-57.

Krishnareddy, S. R., Stewart, B. A., Payne, W. A., Robinson, C. A. and Thomason, R. C. (2006): Tillering in dryland grain sorghum clumps as influenced by light, planting density and geometry, Southern Conservation Systems Conference, Amarillo TX, June 26-28, p:162-170.

Kucuksemerci, O. (2017): The effects of the plant density on syrup yield and quality characteristics of sweet sorghum grown as second crop**,** Canakkale Onsekiz Mart University, Graduate School of Natural and Applied Sciences, M. Sci. Thesis in Field Crops Science, 30 p.

Lafarge, T. D., Broad, I. J. and Hammer, G. L. (2002): Tillering in grain sorghum over a wide range of population densities: Identification of a common hierarchy for tiller emergence, leaf area development and fertility, Ann. Bot, 90:87-98.

Mahmood, A. (2012): Performance of sorghum (*Sorghum bicolor* L. Moench) as an energy crop for biogas production, Justus Liebig University, Institute of Agronomy and Plant Breeding I, Ph. D. Thesis, 119p.

Mahmoud, E. A., Ramadan, B. S. H., Bekheet, M. A. and Gomaa, M. A. (2013): Effect of nitrogen fertilization and plant density on productivity and quality of sweet sorghum, American-Eurasian J. Agric. & Environ. Sci., 13(5):654-659.

Mahmoud, E. A., Ramadan, B. S. H., Hassanien, M. A. and Abo E-Ella, M. G. (1999): Effect of nitrogen fertilization and planting, density on yield and quality on yield and quality of sweet sorghum (*Sorghum bicolor* L.), Egypt J. Appl. Sci., 14(10): 96-115.

Mekdad A. A. A. and Rady, M. M. (2016): Productivity response to plant density in five *Sorghum bicolor* varieties in dry environments, Ann. Agric. Crop Sci. 1(2):1009-1015.

Mokadem S. H. A. (1994): Effect of plant population on some sweet sorghum varieties, Minia J Agric Res Dev., 16: 637-649.

Moosavi, S. G., Seghatoleslami, M. J. and Moazeni, A. (2012): Effect of planting date and plant density on morphological traits, LAI and forage corn yield in second cultivation, Int. Res. J. Basic Appl. Sci., 3(1):57-63.

Reddy, B. V. S., Ramesh, S., Reddy, P. S., Ramaiah, B., Salimath, P. M. and Kachapur, R. (2005): Sweet sorghum-a potential alternate raw material for bio-ethanol and bioenergy, International Sorghum and Millets Newsletter, 46, 79-86pp.

Rutto, L. K., Xu, Y., Brandt, M., Ren, S. and Kering, M. K. (2013): Juice, ethanol, and grain yield potential of five sweet sorghum (*Sorghum bicolor* [L.] Moench) cultivars, J Sustain Bioenergy Syst. 3:113-118.

SAS Institute (1998): INC SAS/STAT user’s guide release 7.0, Cary, NC, USA.

Stell, R. G. D., Torrie, J. A. and Dickey, D. A. (1997): Principles and Procedures of Statistics, A Biometrical Approach 3rd Edi. Mc Graw Hill Book. INC. NY.

Turgut, I., Bilgili, U., Duman A. and Acikgoz, E. (2005): Production of sweet sorghum (*Sorghum bicolor* L. Moench) increases with increased plant densities and nitrogen fertilizer levels, Acta Agr Scand B-SP Sci, 55(3):236-240.

Zand, N. and Shakiba, M. R. (2013): Effect of plant density and nitrogen fertilizer on some attribute of grain sorghum (S*orghum bicolor* (L.) Moench), Int. J. Adv. Bio. and Biomed. Res, 1(12):1577-1582.

COMPARATIVE VALUE OF DRY MATTER YIELD OF GRASSES AND LEGUMES OF TEMPORARY GRASSLANDS UNDER DIFFERENT CUTTING REGIME*

UPOREDNA VRIJEDNOST PRINOSA SUHE MASE TRAVA I LEGUMINOZA PRIVREMENIH TRAVNJAKA PRI RAZLIČITOM REŽIMU KOSIDBE

Muamer Bezdrob[1], Aleksandar Simić[2]

Original scientific paper

Summary

Permanent and temporary grasslands are the most important source of roughage in Bosnia and Herzegovina as they account for more than 50% of total agricultural area. Unfortunately, permanent grasslands of B&H are low-yield (0.5-1.5 t ha^{-1}), and forage collected from them is of low to medium quality. Temporary grasslands are more productive (2.5-3.5 t ha^{-1}), but forage quality varies depending on the botanical composition and stage of plants growth at cutting.
The objective of this study was to determine the influence of cutting regimes on dry mass productivity of different types of grasses and legumes in the plant community.
The three year research on dry mass yields shows that the cutting of legumes at blooming stage achieved a significantly higher yield compared to the stages of grazing imitation and budding of legumes, and therefore the cutting regime significantly influences the production of dry mass, irrespective of the botanical composition. Overall, the most productive mixture regardless of the cutting regime was S3, composed of bird's-foot trefoil, red clover, Italian ryegrass, timothy-grass and orchard grass, 20% each. Value of dry mass of this mixture was 20.35 t ha^{-1} (pasture imitation phase); 21.53 t ha^{-1} (legume budding phase), and; 25.64 t ha^{-1} (legume flowering phase.)

Key words: *temporary grassland, cutting regime, dry mass yield.*

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Faculty of Agriculture and Food Sciences of University of Sarajevo, Zmaja od Bosne 8, 71 000 Sarajevo, Bosnia and Herzegovina
[2] Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Zemun Serbia
Correspondence: Muamer Bezdrob, Faculty of Agriculture and Food Sciences of University of Sarajevo, Zmaja od Bosne 8, 71 000 Sarajevo, Bosnia and Herzegovina. E-mail: m.bezdrob@ppf.unsa.ba

Rezime

Travnjaci, trajni i privremeni, predstavljaju u Bosni i Hercegovini najvažniji izvor kabaste krme, jer zauzimaju preko 50% ukupnih poljoprivrednih površina. Prirodni travnjaci BiH su niskoproduktivni (0,5-1,5 t ha^{-1}), a krma dobijena sa tih površina ima loš do osrednji kvalitet. Privremeni travnjaci su nešto produktivniji (2,5-3,5 t ha^{-1}), ali im kvalitet krme jako varira u zavisnosti od sastava i vremena kosidbe, imajući u vidu fazu razvoja biljaka pri kosidbi.
S obzirom na navedene činjenice, cilj ovog rada bio je utvrđivanje uticaja režima kosidbe na produktivnost suhe mase različitih vrsta trava i leguminoza u biljnoj zajednici, sa posebnim naglaskom na različit režim kosidbe. Trogodišnja istraživanja prinosa suhe mase su pokazala da je pri režimu kosidbe u fazi cvatnje leguminoza postignut značajno veći prinos u odnosu na fazu imitacije ispaše i fazu pupanja leguminoza, te stoga režim kosidbe utiče značajno na produkciju suhe mase, neovisno o botaničkom sastavu. Sveukupno najproduktivnijom neovisno o režimu kosidbe pokazala se smjesa (S3) sastavljena iz smiljkite, crvene djeteline, talijanskog ljulja, mačijeg repka i ježevice sa po 20% u smjesi. Vrijednost prinosa suhe mase ove smjese iznosio je 20,35 t ha^{-1} (faza imitacije ispaše); 21,53 t ha^{-1} (faza pupanja leguminoza) te 25,64 t ha^{-1} (faza cvatnje leguminoza).

Key words: *privremeni travnjaci, režim kosidbe, prinos suhe mase.*

INTRODUCTION

Temporary grassland, commonly known as grass-legume mixture, is usually composed of different types of grasses and legumes. The advantage of such crops is reflected not only in higher biomass yield and better forage quality but also in certain environmental benefits such as soil conservation and improvement of its fertility (thanks to legumes), protection of water against nitrogen leaching, and the like.
In addition to high biomass production, one of the reasons for growing grass-clover mixtures is to better exploit the soils that are not suitable for legume monocropping, since the mixtures are much easier to use for grazing and storing silage. The mixtures also contribute to soil fertility improvement as they leave a large amount of root mass whose decomposition produces humus which has a beneficial effect on the soil structure and water-air regime.
Although potentially high-yielding, sown grasslands often fail to achieve the yields expected given the biological potential of sown species. The reasons may be different, starting from inadequate environmental and soil conditions, the applied agricultural practices and particularly the composition of mixtures at sowing, the subsequent development and representation of individual species in the structure of grass lawn. Lazarević *et al.* (1998) and Kessler and Lehman (1998) argue that proper choice of grasses and legumes suited for specific soil conditions and the expected

duration of crops life is essential for achieving high and stable yields, because in this way the best results can be achieved.

Our temporary grasslands usually contain legumes such as red clover *(Trifolim pratense* L.*)* and bird's-foot trefoil *(Lotus corniculatus* L.*)*, and grasses such as Italian ryegrass *(Lolium italicum* L.*)*, orchard grass *(Dactylis glomerata* L.*)* and timothy-grass *(Phleum pratense* L.). Ready-made mixtures of grasses and legumes that can be found on the market often contain significantly more species with essentially different morphological and biological properties. Biologically, each species and even specific types of the same species have different requirements for environmental conditions, different sprouting and development rate, etc., which makes the growth and development of specific species within a plant community more complex and their representation in the yield very questionable and uncertain, regardless of the planned share at establishing the crop (sowing).

More frequent cutting (fewer days between defoliations) results in lower yield of dry matter and better quality of the fodder (Turner *et al*., 2006). In this regard, the results presented by Brink *et al*. (2010) also show that in less frequent cutting regime (three times a year) it is possible to expect higher annual yield of dry matter compared to frequent cutting (six times a year).

In addition to fertilization, the yield and quality of plant mass of the sown grassland are also influenced by the time of defoliation and the way of defoliation in terms of whether the grassland was cut and when or it was grazed by different species of domestic animals (Andreata-Koren *et al*., 2009).

The significance of the impact of plant development stage at cutting, both in terms of the yield and the quality of fodder, is also pointed out by the research (Sima and Pacurar, 2002; Alibegović-Grbić *et al*., 2004).

Given the above stated facts, the purpose of this study was to determine the influence of different cutting regimes on the productivity of dry mass of various types of grasses and legumes in the plant community.

MATERIAL AND METHODS

The experiment, in which a three-year research was carried out, was set up on the experimental field Butmir (2011-2013). The field experiment was set in randomized blocks with four repetitions. The basic plot size was 5 m². The sowing was done manually, in rows with 12.5 cm spacing between the rows. Seed rate was 15.00 kg ha^{-1} for red clover (variety Viola), 12.00 kg ha^{-1} for bird's-foot trefoil (variety Bosnalotus), 25.00 kg ha^{-1} for Italian ryegrass (variety Tur), timothy-grass (variety SK-45) 12.00 kg ha^{-1} and orchard grass (variety Fala) 30.00 kg ha^{-1}. Mineral fertilizer NPK (15:15:15) was applied at pre-sowing preparation of the soil in the amount of 250 kg ha^{-1}. Soil characteristics of the experimental field were as follows: pH- 5.0 (in KCl), pH- 5.7 (in H_2O); humus content 2.6%, which puts it in the class of medium humus content soils; poor to medium content of phosphorus amounting 9.8 mg 100 g^{-1} of soil; according to

the content of potassium (20.8 mg 100 g^{-1} of soil) this soil belongs to the class of medium potassium content soils.
The experiment investigated: **(A)** two types of legumes and three types of grasses, in four variants of the composition of temporary grassland, as follows:

1. Red clover (*Trifolium pratense* L.) (40%) - (Mixture-S1)
   Italian ryegrass (*Lolium italicum* L.) (20%)
   Timothy-grass (*Phleum pratense* L.) (20%)
   Orchard grass (*Dactylis glomerata* L.) (20%)

2. Bird's-foot trefoil (*Lotus corniculatus* L.) (40%) - (Mixture -S2)
   Italian ryegrass (*Lolium italicum* L.) (20%)
   Timothy-grass (*Phleum pratense* L.) (20%)
   Orchard grass (*Dactylis glomerata* L.) (20%)

3. Red clover (*Trifolium pratense* L.) (20%) - (Mixture -S3)
   Bird's-foot trefoil (*Lotus corniculatus* L.) (20%)
   Italian ryegrass (*Lolium italicum* L.) (20%)
   Timothy-grass (*Phleum pratense* L.) (20%)
   Orchard grass (*Dactylis glomerata* L.) (20%)

4. Italian ryegrass (*Lolium italicum* L.) (33.3%) - (Mixture -S4)
   Timothy-grass (*Phleum pratense* L.) (33.3%)
   Orchard grass (*Dactylis glomerata* L.) (33.3%)

(B) Three variants of defoliation (cutting)
a) Cut at a height of 20 cm (imitation of grazing), b) Cut at budding stage of the legume component, and c) Cut at flowering stage of the legume component.
The total yield of dry mass was determined based on the yield of green mass established at defoliation (cutting) and the content of dry matter. The dry matter content was determined by drying the taken samples in the dryer to a humidity of 13 to 15%. The results were subjected by the SPSS 22 program and tested with the LSD test.

RESULTS AND DISCUSSION

Comparative value of dry mass yield under different cutting regime in 2011

The comparative value of the dry mass yield under the different cutting regimes in the year of sowing shows that, on average, the highest annual yield was achieved (Table 1) under the cutting at legume budding stage (4.55 t ha^{-1}), followed by grazing imitation stage (4.50 t ha^{-1}), while the lowest one was at the legume flowering stage (4.19 t ha^{-1}). One of the reasons for the low dry mass yield in the legume flowering stage was the rather unfavorable weather followed by the lack of moisture and high air

temperatures during the period of formation of the second cut under this cutting regime, which resulted in having only one cut. Due to earlier cutting, and therefore better conditions for regeneration, two cuts were obtained at the imitation of grazing and legume budding stage, each.
Although some differences were identified between different cutting regimes, they were not statistically significant.

Tab. 1. Comparative value of the dry mass yield (t ha^{-1}) in 2011 under different cutting regimes

Mixture variants (Temporary grassland)	**Cutting regime**		
	Imitation of grazing	**Budding**	**Flowering**
Mixture (S1)	4.77	5.17	4.03
Mixture (S2)	4.96	4.36	3.82
Mixture (S3)	4.85	5.09	4.89
Mixture (S4)	3.44	3.58	4.04
Average	**4.50 ns**	**4.55 ns**	**4.19ns**

^{ns} There is no significant differences among cutting regimes (P<0.05).

Observed by the mixtures and cutting regime, the highest yield of dry mass was achieved by mixture S1 (5.17 t ha^{-1}) at the legume budding stage, which is by 0.40 t ha^{-1} more than the dry mass yield at the stage of imitation of grazing and by 1.14 t ha^{-1} more compared to the cutting at the flowering stage of legumes. The lowest yield of dry mass, at all cutting stages, was recorded in mixture S4 consisting only of grasses.

Comparative value of dry mass yield under different cutting regime in 2012
The cutting regime had a significant impact on dry mass yields in all the examined mixtures (Table 2), irrespective of the botanical composition. Under the cutting regime at the stage of flowering of legumes, a statistically higher dry mass yield was achieved compared to the yields achieved at the stage of budding of legumes or the stage of imitation of grazing. In addition, under the cutting regime at the stage of budding of legumes, a considerably higher yield of dry mass was achieved compared to the one at the stage of imitation of graze, except for mixture S4. Thanks to more favorable weather conditions and more balanced distribution of rainfall as well as to a better development of the plants, the dry mass yields and number of cuts in 2012 were considerably higher compared to the year 2011.
The highest annual yield of dry mass in 2012 was achieved by mixture S1 (10.21 t ha^{-1}) under the cutting regime at the flowering stage of legumes, while at the stage of imitation of grazing and legumes budding stage it was the mixture S3 (6.17 t ha^{-1} and 8.18 t ha^{-1}). On the other side, the lowest annual yield was recorded in mixture S4 under all cutting regimes, ranging from 2.76 t ha^{-1} in the budding stage of legumes to 3.39 t ha^{-1} in the flowering stage of legumes.

Tab. 2. Comparative value of the dry mass yield (t ha^{-1}) in 2012 under different cutting regimes

Mixture variants (Temporary grassland)	**Cutting regime**		
	Imitation of grazing	**Budding**	**Flowering**
Mixture (S1)	5.72c	7.44b	10.21a
Mixture (S2)	4.70c	6.26b	8.21a
Mixture (S3)	6.17c	8.18b	9.42a
Mixture (S4)	2.98b	2.76b	3.39a
Average	**4.89**	**6.16**	**7.80**

[abc] Values marked with different letters are showing significant differences among cutting regime (P<0.05).

Comparative value of dry mass yield under different cutting regime in 2013

In the last year of the research (2013), the highest average annual yield of dry mass (Table 3), in all of the examined mixtures, was achieved under the cutting regime at the flowering stage of legumes (10.67 t ha^{-1}), followed by the imitation of grazing stage (8.61 t ha^{-1}), while the lowest one was recorded at the budding stage of legumes (8.10 t ha^{-1}). Cutting at the flowering stage of legumes, irrespective of the variant of the mixture, has shown statistically significantly higher yield of dry mass compared to the stages of budding of legumes and imitation of grazing. No significant difference in the yield of dry mass was established between the variants of the first two cutting regimes (imitation of grazing and legume budding stage).

The most productive, under each cutting regime, was mixture S2. Dry mass values for mixture S2 amounted 9.81 t ha^{-1} (budding stage of legumes), then 10.27 ha^{-1} (stage of imitation of grazing), and 12.41 t ha^{-1} in cutting at the flowering stage of legumes.

Tab. 3. Comparative value of the dry mass yield (t ha^{-1}) in 2013 under different cutting regimes

Mixture variants (Temporary grassland)	**Cutting regime**		
	Imitation of grazing	**Budding**	**Flowering**
Mixture (S1)	7.90b	7.78b	11.01a
Mixture (S2)	10.27b	9.81b	12.41a
Mixture (S3)	9.33b	8.26b	11.33a
Mixture (S4)	6.94b	6.58b	7.93a
Average	**8.61**	**8.10**	**10.67**

[ab] Values marked with different letters are showing significant differences among cutting regime (P<0.05).

The lowest productivity in this year, at all cutting stages, was again achieved by mixture S4 whose yield ranged from 6.58 t ha^{-1} (budding stage of legumes) to 7.93 t ha^{-1} (flowering stage of legumes).

Comparative three-year yield of the dry mass under different cutting regimes

The summed three-year results of the dry mass yield study (Table 4) indicate a significant impact of the cutting regime on the productivity of the mixtures, as the highest dry mass yield was achieved at the flowering stage of legumes with the average total yield of all examined mixtures amounting 22.67 t ha^{-1}, followed by the budding stage of legumes with 18.81 t h^{-1} a, and the lowest one at the stage of imitation of grazing with only 18.00 t ha^{-1} of dry mass. The same fact was indicated by the research results of Leto *et al.* (2013), who found that delaying of cutting until the beginning of flowering significantly increases the yield of dry mass in two of three examined cultivars in the year of sowing, as well as in all the cultivars in the last year of research. In all three years of research, at the beginning of flowering, the stalk portion of the plant increased whereas the leaf and flower portions decreased compared to the beginning of budding.

Tab. 4. Comparative value of the three-year dry mass yield (t ha^{-1}) under different cutting regimes

Mixture variants (Temporary grassland)	Cutting regime		
	Imitation of grazing	**Budding**	**Flowering**
Mixture (S1)	18.39b	20.39b	25.25a
Mixture (S2)	19.93b	20.43b	24.44a
Mixture (S3)	20.35b	21.53b	25.64a
Mixture (S4)	13.36b	12.92b	15.36a
Average	**18.00**	**18.81**	**22.67**

[ab] Values marked with different letters are showing significant differences among cutting regime (P<0.05).

On the other hand, the effect of the (botanical) composition on the productivity is clearly pronounced. In our research, the largest three-year total yield of dry mass, irrespective of the cutting regime, was achieved by mixture S3, in which red clover, bird's-foot trefoil, timothy-grass, Italian ryegrass and orchard grass were represented with 20% each. Its total three-year yield of dry mass was: 25.64 t ha^{-1} at the stage of the flowering of legumes; 21.53 t ha^{-1} at the stage of the budding of legumes, and; 20.35 t ha^{-1} at the stage of grazing imitation. Mixture S4 composed of Italian ryegrass, timothy-grass and orchard grass showed the lowest productivity in all three years of the research and therefore could not compete with grass and legume mixtures, particularly not without the application of larger doses of nitrogen fertilizers (Nyfeler *et al.*, 2008). In the second place, by total productivity (25.25 t ha^{-1}), was mixture S1 consisting of red clover (40%) and Italian ryegrass, timothy-grass and orchard grass represented by 20% each at sowing.

CONCLUSION

Based on the three-year results of studying the comparative values of dry mass yields of grasses and legumes of temporary grassland under the different cutting regimes, the following can be concluded:

Three-year results of dry mass yield research show that the cutting regime at the stage of flowering of legumes provided a significantly higher yield of dry mass compared to the stages of grazing imitation or budding of legumes, hence the cutting regime significantly influences the productivity of the dry mass, irrespective of the botanical composition.

The highest three-year yield of dry mass was achieved by mixture S3, composed of bird's-foot trefoil, red clover, Italian ryegrass, timothy-grass and orchard grass (25.64 t ha^{-1} at the flowering stage of legumes; 21.53 t ha^{-1} at the budding stage of legumes, and; 20.35 t ha^{-1} at the stage of grazing imitation).

Mixture S4, consisting of Italian ryegrass, timothy-grass and orchard grass had the lowest productivity in all three years of research and therefore could not compete with grass and legume mixtures.

REFERENCES

Alibegovic-Grbic, S., Civic, H., Cengic, S., Muratovic, S., Dzomba, E. (2004): Effect of weather conditions, stage of plant growth and N application on yield and quality of grassland in Bosnia and Herzegovina. In: Luscher, A. et al. (eds) Land Use Systems in Grassland Dominated Regions. Grassland Science in Europe. Vol. 9. pp. 897-899.

Andreata-Koren, M., Knežević, M., Leto, J., Safner, T., Marijana Ivanek-Martinčić, Zvjezdana Augustinović (2009): Reakcija klupčaste oštrice (*Dactylis glomerata L.*) u travno-djetelinskoj smjesi na gnojidbu dušikom i napasivanje. Mljekarstvo 59 (3), 254-261.

Brink G. E., Casler, M. D., Martin, N. P. (2010): Meadow Fescue, Tall Fescue, and Orchardgrass Response to Defoliation Management. Agronomy Journal 102, 667-674.

Kessler, W., Lehmann, J. (1988): Evaluation of grass/clover mixtures for leys. In Nagy, g and Peto, K. (eds) Ecological Aspects of Grassland Management. Grassland Science in Europe, vol.3. pp. 231-235.

Lazarević, D., Stošić, M., Dinić, B., Terzić, D. (1998): Production of artifical grasslands in lawland araes. Proceedings of 2nd Balkan Symposium on Field Crops, Vol. 2, Novi Sad, p. 485-488.

Leto, J., Perčulija, G., Bošnjak, K., Kutnjak, H., Marina Vranić, Ivana Čačić (2013): Utjecaj bakterizacije, kultivara i stadija zrelosti na prinos i kemijski sastav crvene djeteline. Mljekarstvo, Vol.63. (2), 98-108.

Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E., Luscher, A. (2008): Well-balanced grass-legume mixtures with low nitrogen fertilization can be as productive as highly fertilized grass monocultures. In Hopkins,. et. al. (eds). A Biodiversity and Animal Feed. Future Challenges for Grassland production. Grassland Science in Europe, 13, 197-199.

Sima, N., Pacurar, F. (2002): Quality of forage obtained from mountain pasture as influenced by harvesting phenophase and management. In: Durand, J. L. et al. (eds) Multi-grasslands function. Grssland Science in Europe. Vol. 7. pp. 160-161.

Turner, L. R., Donaghy, D. J., Lane, P. A., Rawnsley, R. P. (2006): Effect of defoliation management, based on leaf stage, on perennial ryegrass (*Lolium perenne* L), prairie grass (*Bromus willdenowii* Kunth.) and cocksfoot (*Dactylis glomerata L.*) under dryland conditions. 2. Nutritive value. Grass and Forage Science 61, 175-181.

YIELD AND QUALITY SELECTED VARIETIES OF LETTUCE (*Lactuca sativa* L.)*

PRINOS I KVALITET ODABRANIH SORTI SALATE (*Lactuca sativa* L.)

Ćerima Zahirović[1], Lutvija Karić[1], Dragan Žnidarčič[2], Senad Murtić[1], Josip Jurković[1]

Original scientific paper

Summary

Lettuce (*Lactuca sativa* L.) is a vegetable rich in vitamins, especially in vitamin C, mineral substances and other bioactive compounds with high antioxidant properties. Taking into consideration the fact that lettuce is the most commonly grown vegetable in the world, there is a large selection of varieties. Main goals of modern, contemporary varieties of lettuce are to satisfy particular tastes of consumers, and to make profit for the producer. With correct choice of the variety, the aim is to create balance for both parties.

The aim of research was to determine the contents of vitamin C, phenol, antioxidant capacity, yield and morphological properties in different lettuce varieties. The research was conducted in winter 2016/2017 in greenhouse without heating system, in region of Srebrenik. The experiment was set on split-plot method with three repetitions with varieties of Butterhead (Shangore F1 i Nansen) and Iceberg / Crisphead (Funly F1).

Average mass of lettuce head and hence yield of lettuce, varied significantly between different varieties. On the other side, there were no significant differences in vitamin C, phenol contents and antioxidant capacity.

Key words: *lettuce, variety, yield, phenol, vitamin C, antioxidative capacity*

Rezime

Salata (*Lactuca sativa* L.) je povrće bogato vitaminima, naročito vitaminom C, mineralnim tvarima i drugim bioaktivnim spojevima sa visokim antioksidacijskim svojstvima. Uzimajući u obzir činjenicu da je zelena salata najviše uobičajeno uzgojeno povrće u svijetu, postoji veliki izbor sorti. Glavni ciljevi savremenih sorti zelene salate su zadovoljiti posebne ukuse potrošača i ostvariti dobit proizvođaču. S pravim izborom sorte postiže se ravnoteža za obje strane.

Cilj istraživanja bio je utvrđivanje sadržaja vitamina C, ukupnih fenola, antioksidacijskog kapaciteta, prinosa i morfoloških karakteristika kod različitih sorti

---

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Faculty of Agriculture University of Sarajevo, Zmaja od Bosne 8, 71 000 Sarajevo, Bosnia and Herzegovina
[2] Biotechnical Faculty of University Ljubljana, Jamnikarjeva 101, 1 000 Ljubljana, Slovenia

zelene salate. Istraživanje je provedeno tijekom zimskog perioda 2016/2017 godine u plastenicima bez grijanja, na području Srebrenika. Ogled je postavljen split-plot metodom u tri ponavljanja sa sortama glavičaste salate (Shangore F_1 i Nansen) i kristalke (Funly F_1).
Prosječna masa glavice salate i prinos značajno su varirali između različitih sorti. S druge strane, nije bilo statistički značajnih razlika u sadržaju vitamina C, fenola i antioksidacijskog kapaciteta.

Ključne riječi: *sorta, prinos, fenoli, vitamin C, antioksidacijski kapacitet.*

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is a highly valued vegetable in human nutrition not only for its richness in minerals, vitamins and other bioactive compounds with high antioxidant properties but also for the fact that nowadays it is produced all year round, and consumed fresh so that all the ingredients stay intact. The nutrient and phytochemical content varies depending on lettuce type (Mou, 2005; USDA, 2005). Consumption of antioxidants in food via natural sources is good for the prevention of cardiovascular diseases, especially arteriosclerosis (Hu, 2000). Among these, vitamin C and phenol constitute important groups in human diet. Over the last decade, there has been increased interest in plant foods that are rich in health-protecting source of minerals, vitamins and phytochemicals (Veberič *et al*., 2009). Jevrić *et al*. (2017) were concluded Regression coefficient along chlorophyll a, chlorophylla 1 b, and total carotenoids content indicates that they have a greater influence on the antioxidant activity than other phytochemical in lettuce. The production of high quality vegetable is of vital importance to the vegetable industry related to international standards. China produces almost half of the world’s lettuce output, which amounts to 11 million Mt. The total area in Bosnia and Herzegovina was 824 hectares and the total yield was 6102 tons (FAOstat, 2014). Lettuce is grown in Bosnia and Herzegovina for local consumption and export. Also, lettuce is a vegetable that can successfully be grown in almost all sowing periods. Greenhouse production in Bosnia and Herzegovina in the last twenty years is experiencing significant expansion. Taking into consideration the fact that greenhouse lettuce growing plays an important role both from the perspective of the farm as well as from that of the entrepreneur in Bosnia and Herzegovina, the aim of the research was to distinguish and to measure the differences in morphologic, productive and qualitative characteristics of the three the most planted lettuce sorts in greenhouses of Bosnia and Herzegovina.

MATERIALS AND METHODS

The research was conducted in winter 2016/2017 in greenhouse without heating system, in region of Srebrenik. The experiment was set on split-plot method with three repetitions with varieties of Butterhead (Shangore F1 i Nansen) and Iceberg /

Crisphead (Funly F1). The size of each plot was 1 m × 1 m. The lettuce was produced from seedlings planted on black PE foil, at a spacing of 25 x 25 cm. Each plot contained 16 lettuce plants. During the production cycle, all standard measures for the cultivation of lettuce on foil were employed. At technological maturity lettuce samples were collected for analyses.
The analyses of nutrients contents were performed after harvest. A mixed sample was done from three plants of each repetition.

- Lettuce plant diameter was measured only from the part of the plant that we can eat.
- Lettuce heads were weighed on digital scales. Average weight of heads and yield per square meter were calculated from the measured values.
- To determine dry matter weight, all of the samples were placed separately into paper bags and dried in a forced air flow oven at 105 °C until reaching constant weight, obtained using a precision balance.
- The content of L-ascorbic acid was determined using the Tillman's method (AOAC, 2002). Three parallel titrations were performed for each sample. The determined volume of the lettuce extract (5.00 cm^3 of extract + 5 cm^3 of the mixture of HPO_3 and glacial CH_3COOH) was titrated with Tillman's reagent until it turned into light pink color that persisted for about five seconds. The average values of the volumes of three titrations were taken for calculation of the vitamin C content in lettuce.
- Total phenolic content was measured using the Folin–Ciocalteu colorimetric method (Ough and Amerine, 1988). After proper dilution, the diluted extract, 0.25 ml used for the FRAP assay was mixed with 15 ml distilled water, 1,25 ml of Folin–Ciocalteu reagent (Sigma Chemical Co, St Louis, MO), and incubated for 3 minutes at 23 °C. Then 3,75 ml of Na_2CO_3 was added. Absorbances at 765 nm were recorded for the mixtures after 30 minutes incubation at 50 °C.
- Antioxidant capacity in the supernatants was determined using the ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996). Briefly, 100-μl samples were mixed with 3 ml of FRAP reagent. The FRAP reagent was prepared daily by combining 300 mM sodium acetate buffer (pH 3.6), 10 mM 2,4,5,-tripyridyl-Striazinein 40 mM HCl, and 20 mM $FeCl_3$ in the ratio of 10:1:1 (v:v:v). The mixture was incubated at 23 °C for 30 minutes, then absorbance at 593 nm was measured with a spectrophotometer FRAP values were calculated from $FeSO_4$ standard curves, and expressed as μmol g^{-1} FRAP values.
- Average values of the parameters in the study were statistically processed with computer, using software Excel. Data analysis was performed using the Analysis of Variance (ANOVA) and Least Significant Differences (LSD).

RESULTS AND DISCUSSION

Table 1 shows effect of sorts on morphologic and productive characteristics of lettuce. The differences between sorts in morphologic parameters weren't statistically significant. The highest plant diameter was obtained from Funly (32,83 cm), which was followed by Nansen (32,66). The productive parameters which showed significant differences between sorts are average mass of the formed head and yield.

Table 1. Sorts effect on leaf yield and yield contributing characteristics of the lettuce.

Parameters / Sorts	Spread diameter (cm)	Leaf yield plant (kg)	Yield kg m^{-2}
Shangore	30.33^{a}	0.37^{a}	5.95^{a}
Nansen	32.66^{a}	0.24^{b}	3.86^{b}
Funly	32.83^{a}	0.26^{b}	4.18^{b}

The highest leaf yield per plant was obtained from Shangore (0.37 kg) and lowest was found in Nansen (0.24 kg). The highest leaf yield per square meter had Shangore (5.95 kg), which was statistically different from other sorts. The lowest yield (3.86 kg m^{-2}) was obtained in the Nansen variety, which also had the smallest head. The factors such as cultivation season, weather conditions and cultivar influenced the formation of phytomass of leaf rosette, which affected its total weight. Koudela and Petříková (2008) also reported that the spread diameter, average weight of heads and yield are affected by sort. These results correspond to the results listed by (Govedarica – Lučić and Perković, 2013; Custic *et al*., 1994; Todorović *et al*., 2012).

Table 2. Sorts effect on qualitative characteristics of the lettuce.

Parameters / Sorts	Dry matter, %	Vitamin C mg/100 g FW	Total phenolic content mg/100 g FW	Antioxidant capacity µmol Fe^{2+} /100 g FW
Shangore	5.81^{a}	6.24^{a}	84.66^{a}	821.16^{a}
Nansen	7.09^{b}	5.41^{a}	82.98^{a}	1008.79^{a}
Funly	6.70^{b}	5.82^{a}	93.80^{a}	1026.01^{a}

Researched parameters and established differences in qualitative analyses didn't have significant meaning except ingredients of dry weight. The levels of dry matter content were statistically smaller in Shangore (5.81%), than in other sorts. The highest % of dry matter was observed in Nansen (7.09%), which was followed by Funly (6.70%). Leclerc *et al*. (1990) showed a negative correlation between dry matter content and the weight of lettuce.

Lettuce cultivars with higher content of vitamin C may be a good source of antioxidants, especially in winter, when there is a deficit in the consumption of fresh vegetables. The results on the L-ascorbic acid content in several types of lettuce, expressed in mg/100 g of fresh lettuce, are presented in table 2. Comparing the three sorts of lettuce, the highest content of vitamin C (6.24 mg/100 g FW) was found in Shangore and the lowest in Nansen (5.41 mg/100 g FW). The differences among cultivars were not statistically significant. Significant differences in vitamin C content among lettuce cultivars were found by Petříková and Pokluda (2003) and Mou (2005). The difference in the vitamin C content was induced by lettuce genotype (Llorach *et al*., 2008).

The results show that lettuce extracts have high content of total phenols and antioxidant capacity, which has been established by other authors in case of other lettuce varieties (Ozgen and Skerci, 2001; Gan and Azrina, 2016). The highest content of total phenols was in the Iceberg lettuce variety Funly (93.80 mg/100 g FW), and the lowest was in the Nansen lettuce (82.98 mg/100 g FW). Iceberg lettuce Funly also had the highest antioxidant capacity (1026.01 μmol Fe^{2+} /100 g FW) than Butterhead lettuce Shangore (821.16) and Nansen (1008.79). The total content of phenols and antioxidant capacity in the research did not represent the significant level of differences among researched varieties.

CONCLUSIONS

The morphologic and productive parameters of lettuce and the content of selected substances ranged as follows: spread diameter of head (30.33 to 32.83 cm),weight of leaf rosette (0.24 to 0.37 kg), yield (3.86 to 5.95 kg m^{-2}), dry matter (5.81 to 7.09%), vitamin C (5.42 to 6.24 mg/100g FW), phenols (82.98 to 93.80 mg/100 g FW) and antioxidant capacity (821.16 to 1026.01μmol Fe^{2+} /100 g FW).

Average mass of lettuce head, hence yield of lettuce and ingredients of dry weight varied significantly between different varieties. On the other side, there were no significant differences in vitamin C, phenol contents and antioxidant capacity.

The presented research of the content antioxidant components (vitamin C, total phenols) and antioxidant activity of three lettuce sorts confirmed the values of lettuce in everyday nutrition. Iceberg lettuce Funly had higher antioxidant capacity and phenolic content than Butterhead lettuce Shangore and Nansen. The tested varieties in protected areas can be successfully grown during the winter period.

REFERENCES

AOAC (2002): Official methods of analysis (17th ed.). Washington, DC. Association of Official Analytical Chemists.

Benzie, I. F. F., Strain, J. J. (1996): Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Anal Biochem, 239: 70-76.

Custic, M., Poljak, M. and Cosic, T. (1994): Nitrate content in leaf vegetables related to nitrogen fertilization in Croatia, Acta Horiculturae, 371: 407-412.

FAO (2014): http://www.fao.org/faostat/en/#data/QC 07.09.2017.

Gan, Y. Z., Azrina, A. (2016): Antioxidant properties of selected varieties of lettuce (*Lactuca sativa* L.) commercially available in Malaysia. International Food Research Journal, 23(6), 2357-2362.

Govedarica-Lučić, A. and Perković, G. (2013): Uticaj sorte i načina proizvodnje na prinos i kvalitetne osobine salate proizvedene u zimskim uslovima. Acta agriculturae Serbica, 18: 35.

Hu, F. B. (2000): Dietary pattern analysis: a new direction in nutritional epidemiology. Current Opinion in Lipidology, 13: 3-9.

Jević L. R., Karadzić M. Z., Podunavac- Kuzmanović S. O. (2017): New guidelines for prediction of antioxidant activity of *Lactuca sativa* L. varieties based on phytochemicals content and multivariate chemometrics. J Food Process Preserv. 2017;00:e13355. https://doi.org/10.1111/jfpp.13355

Koudela, M., Petříková, K. (2008): Nutrients content and yield in selected cultivars of leaf lettuce (*Lactuca sativa* L. var. *crispa*). Hort. Sci. (Prague) 35, (3): 99–106.

Leclerc, J., Reuillie, M. J., Miller, M. L., Lefebvere, J. M., Joliet E., Autissiere N., Martinez Y., Peret, A. (1990): Effect of climatic conditions and soil fertilization on nutrient composition of salad vegetables in Burgundy. Sciences des Aliments, 10: 633–646.

Llorach, R., Martinez-Sanchez, A., Tomas-Barberan, F. A. (2008): Characterisation of polyphenols and antioxidant properties of five lettuce varieties and ascarole. Food Chemistry A, 108: 1028-1038.

Mou, B. (2005): Genetic varation of beta-carotene and lutein contents in lettuce. J.Am.Soc.Hort Sci, 130: 870-876.

Ough, C. S., Amerine, M. A. (1988): Phenolic compounds, In: Methods for analysis of musts and wines, John Wiley & Sons, Inc, New York, USA.

Petříková, K., Pokulda, R. (2003): Influence of variety and cultivation on the nutritional value of lettuce In: Quality of crop production: the present and perspectives of the EU. Proceedings of the Czech-Slovak Conference, Praha, VÚRV: 123-126.

Todorović, V., Gavrić Rožić, A., Markvić, S., Đurovka, M., Vasić, M. (2012): Uticaj temperature na ranostasnost i prinos salate gajene u zimskom periodu. Agroznanje, 13 (3): 475-481.

USDA (2005): USDA National Nutrient Database for Standard Reference, Release18. Nutrient Data laboratory Home Page, http://www.nal.usda.gov/fnic/foodcomp.

Veberič, R., Jakopič, J., Štampar, F., Schmitzer, V. (2009): European elderberry (*Sambucus nigra* L.) rich in sugars, organic acids, anthocyanins and selected polyphenols. Food Chem, 114: 511-515.

THE EFFECTS OF DIFFERENT GROWING TECHNIQUES ON YIELD AND QUALITY OF TOMATO (*Lycopersicon esculentum* Mill.)*

Dragan Žnidarčič[1], Lutvija Karić[2], Nataša Mirecki[3], Lovro Sinkovič[4]

Original scientific paper

Summary

The effect of various growing methods of tomato (*Lycopersicon esculentum* Mill.) cv. Hector on fruit yield and sensory properties was examined. The growing methods used were: bare soil (BS), black plastic mulch (BPM), bare soil with polypropylene row cover (BS + RC) and black plastic mulch with polypropylene row cover (BPM + RC). The yields of all growing methods were reduced during growing season of extreme rainfall. The results show that the use of BPM + RC resulted in the highest total fruit yield (15.93 t ha^{-1}). By using row cover (BS + RC or BPM + RC) it has been observed that marketable yield could be significantly higher than in the BS and the BPM treatments. Plants grown in the BPM and in the BPM + RC yielded larger number of fruits in comparison to plants in the BS or in the BS + RC. The fruit height and diameter were not affected by the treatments. This study also gives a sensory characterisation of various growing methods as an indicator of suitability for growing and marketing of tomatoes. Tomatoes grown in the BPM + RC resulted in the highest total taste intensity.

Key words: *tomato, black plastic mulch, polypropylene cover, yield, fruit quality, sensory evaluation*

INTRODUCTION

Tomato is one of the most valuable fruit vegetable grown in Slovenia, being grown in almost all home gardens as well as commercial operations. Although in botanical terms, tomato is a berry fruit, it is grown and consumed as vegetable. During 2016, the crop was cultivated over an area of 204 ha (Statistical Office RS, 2017).

Important factors to be considered for growing tomatoes are the variety, selection of site, type of soil, fertilization, stage of harvest and crop rotation. Black plastic mulch has been widely used in the production of fresh market tomatoes (Pan *et al*., 1999). The beneficial responses of tomatoes to black plastic mulch, such as higher total

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Biotechnical Faculty of University Ljubljana, Jamnikarjeva 101, 1 000 Ljubljana, Slovenia
[2] Faculty of Agriculture University of Sarajevo, Zmaja od Bosne 8, 71 000 Sarajevo, Bosnia and Herzegovina
[3] Biotechnical Faculty, University of Podgorica, Podgorica, Montenegro
[4] Agricultural Institute, Ljubljana, Slovenia

yields, earlier production and better fruit quality, have been discussed by many authors (Abdul-Baki *et al*., 1992; Call and Courter, 1989; Decoteau *et al*., 1989). Additional benefits of plastic mulches are their ability to conserve soil moisture, and their potential to direct rain water to the crop plants (Lamont, 1993) and reduce insect and disease problems (Greenough *et al*., 1990). Black plastic mulch was supposed to function as a mechanical barrier preventing a direct contact of the plant with the inoculum of the fungus in the soil (Trdan *et al*., 2001). Last but not least the edible product from a mulched crop such as tomato is cleaner and less subject to rots because soil is not splashed on the fruit.

Row covers are another important component of a tomato production system. Row covers are a flexible transparent covering which is installed over plants to reduce the need for insecticide, increase air temperature around the crop. Furthermore, their usage has been associated with increased plant growth, higher yields and earliness of harvest (Siviero and Biribin, 1997; Wells, 1985; Bonanno and Lamont, 1987; Hemphill *et al*., 1988; Jensen, 1990; Teasdale and Abdul-Baki, 1995).

Although the primary objective of tomato growers is to maximise the harvest of fruit per cultivation area, consumers put a great pressure on growers to improve both tomato yield and quality. In comparison to price, produce quality is a more important factor determining the purchase (Schwartz, 1995). Sensory evaluation consists, on one hand of identifying organoleptic qualities of a product and, on the other hand, of recording the satisfaction of the consumer. In order to attain eating quality, it is important to include all sensory characteristics (Auerswald *et al*., 1999).

Many studies have been conducted with black plastic mulch and row covers as treatments on several vegetable crops (Brown and Channell-Butcher, 1999; Purser, 1994; Gerber *et al*., 1983; Ibarra *et al*., 2001) but information is limited on the effects of these materials on the production and quality of tomato. In the efforts to determine the effects of this type of cropping system, the study was conducted to investigate the use of black plastic mulch alone or together with row covers (polypropylene fibril cover material) in the production of tomato. Hence, an experiment was conducted to compare fruit weight, number of fruits, fruit characteristics and to evaluate the sensory quality of fruits.

MATERIALS AND METHODS

The field plots were established in 2014 at the Experimental Station of the Biotechnical Faculty in Ljubljana. The tomato (*Lycopersicon esculentum* Mill.) used for the study was determinate tomato cv. Hector. Tomato seeds were sown into 72-cell styrofoam trays filled with commercial peat. The trays were placed in a greenhouse. The seedlings were liquid fed once per week using, 0.75 g litre^{-1} N, 0.55 g litre^{-1} P_2O_5 and 1.45 g litre^{-1} K_2O.

Standard agronomic practices such as fertilisation and plant protection measures were applied during the crop period. The plants were grown in a heavy clay loam soil in the second year after farmyard manure fertilization (30 t ha^{-1}). The field preparations began in mid-April when 0.15-metre-high and 1.1-metre-wide beds were created using a rotary tiller cultivator. Granular fertilizer was incorporated at pre-plant (350 kg ha^{-1}, 15N-15P-15K). Tomatoes were established on raised beds by hand transplanting on 21 April. All beds were uniformly irrigated using T-Tape systems that delivered 5 litres min^{-1} of water per 100 m of tubing with emitting orifices spaced at 30 cm intervals. Fertigation was applied once per week irrespective of rainfall, using a 120 mg l^{-1} N, 80 mg l^{-1} P_2O_5 and 120 mg l^{-1} K_2O.

The experimental design was a randomized complete block with treatments arranged factorial and replicated four times. The treatments were as follows: black plastic mulch (BPM), bare soil (BS), BPM with RC (polypropylene row cover) and BS with RC. Each treatment contained 24 plants in triple rows; only the centre row was used for data collection. Black plastic mulch was laid by a mulch applicator to the appropriate treatments. The wire hoops were erected over the appropriate beds and polypropylene (17 g m^{-2}) cover was laid immediately after planting. Approximately 30 days after transplanting the covers were removed.

Tomato fruits, which were handpicked at the pink to full-ripe stage, were selected randomly from each treatment in the centre rows only. Harvesting was carried out once weekly between 25 of June and 15 of August. The total fruit yield, marketable fruit yield, fruit number per plant and fruit diameter and fruit height were determined immediately after each harvest. Non-marketable yield of fruit included cracked and rotten tomatoes.

The sensory quality was evaluated after the last harvest in the middle of August. Sensory analysis is a method used to directly determine quality by means of human senses, independently of instrumental determinations (Stone and Sidel, 1992). Healthy fruits of uniform size (weight about 70 g) were stored at -18 °C for 24 hours. Before the evaluation they were thawed and sorted according to their size. Each tomato was cut in 12 equal segments. An 18-member panel (eight females/ten males, between 20 and 60 years old) was recruited from the staff and the students at Department of Food Science and Technology. From the same tomato, each panellist was given one segment cut in pieces, served in a small cup, and one whole segment. The method was fully described by Haglund *et al*. (1997). This method of evaluation is quantitative meaning that each descriptive criterion for each sample was scored on a scale from 0 to 9 (Guerineau *et al*., 2000). However, these five criteria had also to be summarised as the intensity of the total taste impression. The average rankings from each panellist were used to calculate the final assessment of each fruit treatment.

Data were subjected to analysis of variance, and means were separated by Duncan's multiple rang test at P = 0.05.

Table 1: Descriptive characters of the profile of tomatoes and instructions for the panel

Profile attribute	***Description***
Taste attributes	
Total taste	The characteristic of total taste intensity that occurs in the tomatoes (0 = low taste intensity, 9 = high taste intensity)
Sweetness	The intensity of sweet taste (0 = low sweet taste, 9 = high sweet taste)
Acidulous taste	The intensity of acidulous taste (0 = low acid taste, 9 = high acid taste)
Bitterness	The aftertaste which remains after the tomato has been expectorated. (0 = no bitter taste, 9 = much bitter taste)
Consistency attributes	
Firmness	The force needed to chew the sample 10 times (0 = little force, 9 = much force)
Juiciness	Degree of juiciness perceived while chewing the sample (0 = little juicy, 9 = very juicy).

RESULTS AND DISCUSSION

Yield and fruit characteristics

Fruit yield data of tomato by various growing methods are presented in Table 2. Yield of all tomato growing practises were generally reduced during the exceptionally rainfall growing time. Tomato fruits were exposed to heavy tomato late blight (*Phytophthora infestans* (Mont.) de Bary) pressure.

Table 2: Effects of various growing methods on fruit yield of tomato*

Treatment	Yield (t ha^{-1})		Non-marketable fruits (%)
	Total	Market	
BS	11.65 a	4.27 a	64.66 a
BPM	12.49 a	4.36 a	64.66 a
BS + RC	12.49 a	7.21 b	43.86 b
BPM + RC	15.93 b	6.72 b	50.23 b

*Values in the same column with different letter are significantly different (level of significance 5%)

The cumulative total fruit yield at the end of the experiment per unit area, with the exception of marketable yield, was significantly enhanced by the use of BPM + RC. The total weight yield in BPM + RC extrapolated to 15.93 tonnes per hectare, while the other growing methods produced the equivalent between 11.65 and 12.49 tonnes per hectare. In agreement with other studies on other crops (Purser, 1994; Loy and

Wells, 1982; Soltani *et al*., 1995), this dramatic increase in tomato yield where BPM and RC were used together, is partially due to an increase in air and soil temperatures around the plant-growing environment.
When marketable tomato yields were compared from the various growing methods, it should be noted, however, that the use of row covers significantly increased marketable yield per unit area. Tomatoes grown in BPM + RC and the BS + RC produced higher yields (6.72 and 7.21 t ha^{-1}, respectively) than those grown in BS and the BPM (4.27 and 4.36 t ha^{-1}, respectively).

An increase in percentage of non-marketable fruits and a decrease of marketable yield was observed in the no cover treatments (BS and BPM). The percentage of non-marketable fruits was lower in the row cover treatments (BS + RC and BPM + RC). As in other studies, fruits produced under row covers were less stressed than fruits produced without covers (Abdul-Baki *et al*., 1992; Pan *et al*., 1999).

Table 3: Effects of various growing methods on fruit characteristics of tomato*

Treatment	Fruit $plant^{-1}$ (No.)	Fruit weight $plant^{-1}$ (kg)	Fruit diameter (cm)	Fruit height (cm)
BS	18.08 a	1.29 a	10.06 a	5.58 a
BPM	20.83 b	1.41 a	9.50 a	5.24 a
BS + RC	18.06 a	1.38 a	10.22 a	6.02 a
BPM + RC	20.91 b	1.77 b	10.67 a	5.87 a

*Values in the same column with different letter are significantly different (level of significance 5%)

The growing method had a significant effect on number of fruit per plant. Fruit height per plant was significantly increased by the BPM (20.83) and by the BPM + RC (20.91) as compared to the BS (18.08) and BS + RC (18.06) treatments. The BPM appeared to enhance fruit number but not fruit weight per plant.

The results indicated that the difference in fruit weight per plant between different growing methods was statistically significant only in the BPM + RC treatment (1.77 kg $plant^{-1}$). No significant differences occurred in fruit weight among other treatments. Final cumulative results from all harvests showed that BPM + RC provided an 18-27% increase in fruit weight per plant compared to all other treatments, respectively.

There were only slight differences in the fruit diameter (ranged from 9.50 to 10.67 cm) and fruit height (ranged from 5.24 to 6.02 cm) due to growing methods. These fruit characteristics were not significantly affected by the treatments.

Sensory analyses

In Table 4, we summarised the 6 attributes which had been outlined during the test with significant changes of intensity. For each descriptive criterion per tomato, the average scores given by the panellists were subjected to analyses of variance.

Table 4: Effects of various growing methods on sensory quality of tomato*

Treatment	Attribute					
	Total taste	Sweetnes	Acid. taste	Bitterness	Firmness	Juiciness
BS	5.78 a	7.69 a	4.72 a	5.64 a	7.01 a	6.04 a
BPM	5.64 a	7.21 a	6.02 b	5.42 a	6.12 b	6.12 a
BS + RC	6.02 a	5.48 b	5.87 b	5.76 a	6.05 b	6.05 a
BPM + RC	6.74 b	5.62 b	6.90 c	5.90 a	5.92 b	7.12 b

*Values in the same column with different letter are significantly different (level of significance 5%)

The results from field experiments indicate the importance of growing method on the sensory quality of tomato fruits. Panellists indicated that the total taste intensity was significantly affected by the growing method. Tomatoes grown in the BPM + RC were scored higher for total taste intensity than tomatoes grown in the other treatments. The tomatoes grown in the BS treatment (control treatment) and tomatoes grown in BPM, were significantly sweeter than tomatoes grown in the BS + RC and in the BPM + RC. Panellists showed the most preference for acidulous taste only for the tomato grown in the BPM + RC. The tomatoes of the control treatment (BS) scored the lowest in acidulous test, while tomatoes grown in the other treatments were more acidulous. Sensory analyses of bitterness showed no significant differences between the treatments. Evaluating of the firmness criterion, panellists could detect a significant difference between the growing methods. The tomatoes from BS treatment were firmer compared to the tomatoes from the other treatments. Panellists showed a preference for the juiciness of the tomatoes grown in the BPM + RC compared with other treatments.

The average rankings from each panellist were also illustrated in the form of a ‘spider web’ plot (Figure 1).

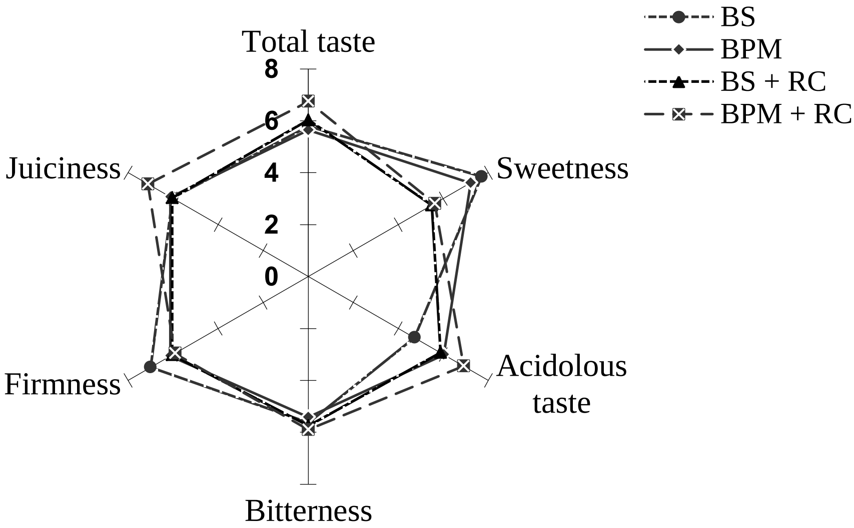


Figure 1: Sensory profiles of different growing method of tomato fruits

CONCLUSIONS

The tomato is one of the most widely accepted vegetables in Slovenia. As more tomatoes are being consumed, the growers have to grow plants with high yield and good quality adapted to their environment. Among the requirements for the optimal growing method, the sensory quality for fresh consumption as well as the marketable yield per unit area are of primary importance.

The results of the presented research show that under Slovenian ecological conditions, black plastic mulches and row covers used alone or in combination offer the grower a management tool which can increase yield and quality. In plants grown with no row cover, the presence of plastic mulch resulted in higher plant yield compared to plants grown in bare soil.

Growing methods used in our experiment also influenced the sensory qualities of tomato. The sensory parameters described in the study must be considered when fresh tomato is handled and marketed.

ACKNOWLEDGMENTS

The authors gratefully acknowledge dr. Åsa Haglund, from Department of Domestic Sciences, Uppsala University for her kind co-operation.

LITERATURE

Abdul-Baki, A., Spence, C., Hoover, R. (1992): Black polyethylene mulch doubled yield of fresh market field tomatoes. HortSci., 27: 787-789.

Auerswald, H., Peters, P., Brückner, B., Krumbein, A., Kuchenbuch, R. (1999): Sensory analysis and instrumental measurement of short-term stored tomatoes (*Lycopersicon esculentum* Mill.). Postharvest Biol. and Technol., 15: 323-334.

Bonnano, A. R., Lamont, W. J., Jr. (1987): Effect of polyethylene mulches, irrigation method, and row covers on soil and air temperature and yield of muskmelon. J. Am. Soc. Hort. Sci., 112: 735–738.

Brown, J. E., Channell-Butcher, C. (1999): Effect of three row covers and black plastic mulch on the growth and yield of 'Clemson Spineless' okra. J. Veg. Crop Prod., 5, 2: 67 – 71.

Call, R. E., Courter J. W. (1989): Response of bell pepper to raised beds, black plastic mulch, spunbonded row cover and trickle irrigation. Proceed. of 21st NAPC: 140–146.

Decoteau, D. R., Kasperbauer, M. J., Hunt, P. G. (1989): Mulch surface colour effects yield of fresh-market tomatoes. J. Am. Soc. Hort. Sci., 114: 216-220.

Gerber, J. M., Brown, J. E., Splittstoesser, W. E. (1983): Intercropping vegetables with plastic mulch and row tunnels. Proc. Natl. Agr. Plastic Conf., 18: 48-55.

Greenough, D. R., Black, L. L., Bond, W. P. (1990): Aluminium-surfaced mulch: An approach to the control of tomato spotted wilt virus in solanaceous crops. Plant Dis., 74: 805-808.

Guerineau, C., Denis, E., Scandella, D., Navez, B., Lancelin, N. (2000): Sensory evaluation of Charentias-type melons: an exploratory tool. Acta Hortic., 510: 487-492.

Haglund, Å., Johansson, L., Gäredal, L., Dlouhy, J. (1997): Sensory quality of tomatoes cultivated with ecological fertilizing systems. Swed. J. Agric. Res., 27: 135-145.

Hemphill Jr., D. D., Crabtree, G. D. (1988): Growth response and weed control in slicing cucumbers under row covers. J. Am. Soc. Hort. Sci., 113: 41- 45.

Ibarra, L., Flores, J., Diaz-Perez, J. C. (2001): Growth and yield of muskmelon in response to plastic mulch and row covers. Sci. Hortic., 87: 139-145.

Jensen, M. H. (1990): Protected cultivation. A global review of plastics in agriculture, Proceed. 11th Int. Congr. on the use of plastics in agriculture. 26 February – 2 March, New Delhi, pp. E-3–E-10.

Lamont, W. J., Jr. (1993): Plastic mulches for the production of vegetable crops. HortTechnol., 3, 1: 35-39.

Loy, J. B., Wells, O. S. (1982): A comparison of slitted polyethylene and spunbonded polyester for plant row covers. HortSci., 17: 405-407.

Pan, H. Y., Fisher, K. J., Nichols, M. A. (1999): Effect of mulch and row covers on yield of process tomatoes. Acta Hortic., 487, 145-150.

Purser, J. (1994): Plastic mulch and row covers to produce warm-season crops in Alaska. Proc. Natl. Agr. Plastic Conf., 24: 49-53.

Schwartz, E. S. (1995): A Simple approach to valuing risky fixed and floating rate debt. J. Financ., 50: 789-819.

Siviero, P., Biribin, R. (1997): Le novità technice nalla coltivazione del pomodoro da industria. L'inf. Agrar., 3: 57-61.

Soltani, N., Anderson, J. L., Hamson, A. R. (1995): Growth and analyses of watermelon plants with mulches and row covers. J. Am. Soc. Hort. Sci., 120: 1001-1009.

Statistical Office RS. (2001): Rapid Reports - provisional data. Ljubl., No. 311, 12. 12. 2001.

Stone, H., Sidel, J. L. (1992): Sensory evaluation practices. Acad. Press, Inc., N. Y., 338 p.

Teasdale, J. R., Abdul-Baki, A. A. (1995): Soil temperature and tomato growth associated with black polyethylene and hairy vetch mulches. J. Am. Soc. Hort. Sci., 120: 848- 853.

Trdan, S., Slapar, A., Rupnik, T., Bobnar, A. (2001): The effectiveness of ecologically acceptable ways of protection of field-grown tomato (*Lycopersicon lycopersicum* (L.) Karsten) from tomato late blight (*Phytophthora infestans* (Mont.) de Bary) in extreme weather conditions. Med. Fac. Landbouww. Univ. Gent., 66,2a: 187-193.

Wells, O. S., Loy, J. B. (1985): Intensive vegetable production with row covers. HortSci., 20: 822–826.

BIOCHEMICAL FRUIT COMPOSITION OF AUTOCHTHONOUS PEAR CULTIVARS FROM BOSNIA AND HERZEGOVINA*

BIOHEMIJSKI SASTAV PLODOVA AUTOHTONIH KULTIVARA KRUŠKE IZ BOSNE I HERCEGOVINE

Senad Memić[1], Amila Vranac[2], Pakeza Drkenda[2], Asima Akagić[2], Osman Musić[2]

Original scientific paper

Summary

The evaluation of fruit biochemical composition of 10 pear cultivars was conducted over two growing seasons. In the study 9 autochthonous pear cultivars and 'Président Druard' as commercial cultivar were included. The experiment was conducted in ex-situ collection located in Srebrenik (North-Eastern Bosnia and Herzegovina). Fruit of similar ripeness stage were analysed in terms of biochemical parameters (soluble solids content, titratable acidity, index of sweetness and total phenolics content). The pear cultivars and growing season had considerable influences on all observed fruit parameters. In this study high correlation coefficients with significant differences were achieved between the total soluble solids and total amount of phenol compounds (r=+0.67) as well as between the titratable acidity and index of sweetness (r=-0.78).
According to our results the cultivar 'Dolokrahan' has the greatest amount of phenols as well as good index of sweetness and soluble solids content. This cultivar could be recommended for fresh consumption and for manufacturing of jams and marmalades. Cultivars 'Takisa' and 'Budaljaca' are achieved the best index of sweetness and could be also use as good raw material in manufacturing of jams and marmalades. The pear cultivar 'Krakaca' could be used for juice production because their richness in soluble solids content and titratable acidity. Standard commercial cultivar 'President Druard' was not emphasized either at one of the analysed parameters of fruit quality.

Key words: *phenolic compounds, Pyrus communis, index of sweetness, soluble solids content, titratable acidity*

Rezime

Evaluacija biohemijskog sastava plodova deset kultivara kruške provedena je tokom dvije uzgojne godine. U radu je posmatrano 9 autohtonih kultivara kruške i 'Druardova maslovka' kao komercijalni kultivar. Istraživanje je provedeno u ex-situ

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Bosmal City Centar, Sarajevo, Bosnia and Herzegovina
[2] Faculty of Agriculture and Food Science, University of Sarajevo, Bosnia and Herzegovina

kolekciji u Srebreniku (sjevero-istočna Bosna i Hercegovina). Analize biohemijskih parametara (rastvorljiva suha tvar, titraciona kiselost, indeks slasti i sadržaj ukupnih fenola) obavljene su na plodovima sličnog stadija zrelosti. Kultivari kruške i uzgojne godine imali su značajan uticaj na sve posmatrane parametre. Visoki koeficijenti korelacije sa značajnim razlikama zabilježeni su između rastvorljive suhe tvari i ukupnih fenola (r=+0.67), te između titracione kiselosti i indeksa slasti (r=-0.78).
Na osnovu rezultata, najveći sadržaj ukupnih fenola, rastvorljive suhe tvari i indeksa slasti zabilježen je kod kultivara ‘Dolokrahan’. Ovaj kultivar može se preporučiti za svježu upotrebu kao i za proizvodnju džemova i marmelada. Kod kultivara ‘Takisa’ i ‘Budaljaca’ zabilježen je najbolji indeks slasti, te se isti mogu koristiti kao dobra sirovina za proizvodnju džemova i marmelada. Kultivar ‘Krakaca’ zbog visokog sadržaja rastvorljive suhe tvari i titracione kiselosti može se preporučiti za proizvodnju sokova. Komercijalni kultivar ‘Duardova malsovka’ se nije isticao niti po jednom analiziranom parametru.

Ključne riječi: *fenolne komponente, Pyrus communis, indeks slasti, rastvorljiva suha tvar, titraciona kiselost*

INTRODUCTION

Territory of current Bosnia and Herzegovina (B&H) was exposed to different civilization influences through history. During past times many “foreign“ germplasm was introduced to this area, same from east and west and at the same time this germplasm took its role into creation of domestic and autochthonous germplasm through spontaneous or planned hybridization or selection. Local and old varieties are generally suppressed from production although their value in terms of biodiversity is extremely high (Ðurić *et al*., 2014). Over the past thirty years, attention has been devoted to the collection, conservation and evaluation of pear diversity, with two main purposes: to conserve a wide genetic variability for breeding programs and to extend the exploitation of old varieties in organic or integrated fruit production (Kellerhals *et al*., 2004). Local and old varieties, as opposed to commercial varieties, often carry specific and uncommon properties that are interesting to a smaller number of consumers. A higher content of phenolics and flavonoids which are produced as a consequence of plant response to a higher exposure to environmental stress is expected from indigenous varieties that are mainly grown without any special plant protection. There is already much evidence that these secondary metabolites have a beneficial effect on health, mainly owing to their ability to scavenge the harmful free radicals, due to which phenols and flavonoids show an increased antioxidant and antimicrobial activity (Percival, 1998; Jakopic *et al*., 2009; Rufino *et al*., 2011). The research of autochthonous pear varieties grown in Bosnia and Herzegovina, conducted for evaluation purposes using pomological and eco-physiological characteristics (Ðurić *et al*., 2009, 2014) or using molecular markers (Gasi *et al*., 2013), showed that cultivars which are traditionally grown in Bosnia and Herzegovina represent an interesting genetic material. The identification and quantification of the biochemical fruit composition of the autochthonous pear cultivars will contribute to the better conservation and increase of whole fruit production as well as to improving of their significance in social development.

MATERIAL AND METHODS

Location and plant material

The research was conducted during 2012 and 2013. Pear fruit for the analysis were taken from collection orchard located in Srebrenik. 9 autochthonous pear cultivars (‘Ahmetova’, ‘Budaljaca’, ‘Dolokrahan’, ‘Hambarka’, ‘Kacmorka’, ‘Krakaca’, ‘Ljeskovaca’, ‘Sarajka’ and ‘Takisa’) and ‘Président Drouard’ as standard commercial cultivar were involved into the analysis. Fruit were harvested at commercial maturity stage, from the previously marked trees.

Determination of chemical parameters

Soluble solid content (SSC) was analysed with a digital refractometer ATAGO Pal-α (Atago CO., LTD., Japan) operating range °Brix 0-85 %. The method for the determination of titratable acidity (TA) utilizes sodium hydroxide (0.1 M NaOH) to neutralize all the titratable protons. The result was expressed as % of the malic acid. Index of sweetness was calculated as the ratio of total soluble solids content and titratable acidity (SCC/titratable acidity).

Extraction procedure

Five grams of grounded fruits was homogenized with a 10 ml extraction solution (methanol containing 3% formic acid and 1% 2,6-di-fert-butyl-4-methylphenol (BHT)) in an ultrasonic ice bath for 1 hour. The mixture was centrifuged at 10 000 rpm for 7 minute at 0 °C. The supernatant was filtered through the Chromafil AO – 45/25 polyamide filter (Macherey – Nagel, Düren, Germany). The obtained fruit extract was used for total phenols analyses, and it was made in 3 replications.

Determination of total phenols

Total phenols were determinated spectrophotometrically by UV-VIS 1700 (Shimadzu, Japan). The content of total phenols of pear fruits was determined using modified colorimetric Folin-Ciocalteau method at wavelength λ= 765 nm, with Folin-Ciocalteau reagent and gallic acid as a standard to produce the calibration curve (Dewanto *et al*., 2002). Results were expressed as mg of gallic acid equivalents (GAE) per kg of fresh weight.

Statistical analysis

Analysis of the fruit quality was done on a sample of 30 fruits in tree repetitions. The data were analysed by calculating the analysis of variance and differences are tested by Tuckey test ($p \leq 0.05$). Principal component analysis was applied to standardized variables. The principal components scores were plotted for individual observations in relation to the most significant axes whose eigenvalues were greater than 1.0.

RESULTS

A statistically significant difference was observed for all examined pomological characteristics. The highest soluble solids content was noted in cultivar ‘Dolokrahan’ (21.75 °Brix), while the smallest was recorded in cultivars ‘Kacmorka’ (16.10 °Brix), ‘Ljeskovaca’ (16.18 °Brix), ‘Badaljaca’ (16.30 °Brix) and ‘Sarajka’ (16.60 °Brix) (Table 1). According to soluble solids content Đurić *et al*. (2014) classified pear genotypes in three groups: i) genotypes with total soluble solids content in fruit flesh juice that is on average level for the species *Pyrus* sp. - from 12.30 to 13.29%; ii) genotypes with increased total soluble solids content in fruit flesh juice - from 13.76 to 15.90%; iii) genotypes with high total soluble solids content in fruit flesh juice - from 15.92 to 19.72%. Analysis of soluble solid content in fruit of analysed genotypes (Table 1) showed that content of soluble solids is above the average level for *Pyrus sp.* In comparison with an overview of pear cultivars from Turkey, where the soluble solids content ranged from 7–15 °Brix (Bostan, 2009), it can be said that soluble solids content in analysed cultivars from B&H (15.20–22.20 °Brix) is greater. Soluble solids content is a very important characteristic of fruit quality for consumers. The content of total soluble solids in pear cultivars from Macedonia varied in a similar range between 12.8° and 18.3 °Brix, neglecting slight variations that result from different varieties and climatic conditions (Selamovska *et al*., 2014) and 14–18.9 °Brix (Selamovska *et al*., 2013). Genotypes with high soluble solids content in fruit flesh juice are also genotypes that are mostly used for liquor (brandy) production.

Table 1. Soluble solid content (°Brix) of evaluated pear cultivars (mean values and standard deviation)

Cultivars	Year 2012		Year 2013		Average	
	Mean	**Sd**	**Mean**	**Sd**	**Mean**	**Sd**
‘Ahmetova’	15.20^{e}	0.26	18.30^{b}	0.10	16.75^{c}	1.71
‘Budaljaca’	16.70^{d}	0.20	15.90^{cd}	0.20	16.30^{c}	0.47
‘Dolokrahan’	22.20^{a}	0.40	21.30^{a}	0.82	21.75^{a}	0.76
‘Président Drouard’	16.60^{d}	0.10	19.13^{b}	0.06	17.87^{c}	1.39
‘Hambarka’	21.00^{b}	0.10	16.03^{c}	0.80	18.52^{bc}	2.77
‘Kacmorka’	17.13^{d}	0.15	15.07	0.12	16.10^{c}	1.14
‘Krakaca’	20.87^{b}	0.12	20.23^{ab}	0.15	20.55^{ab}	0.37
‘Ljeskovaca’	18.03^{c}	0.15	14.33^{d}	0.21	16.18^{c}	2.03
‘Sarajka’	16.43^{d}	0.40	16.77^{c}	0.12	16.60^{c}	0.32
‘Takisa’	17.93^{c}	0.29	18.07^{b}	0.40	18.00^{bc}	0.32
Average	18.21^{x}	2.26	17.51^{y}	2.22	17.86	2.25

a-f - different letters indicate statistically significant differences between cultivars at $p \leq 0.05$
x, y - indicate statistically significant differences between years at $p \leq 0.05$

The highest average titratable acidity was noted in cultivar ‘Krakaca’ (0.51 %), while the smallest (0.09 %) was recorded in both examined years in cultivar ‘Takisa’ (Table 2). The available literature data originating from a pear growing region in Turkey (Ozturk *et al*., 2009), and Bosnia and Herzegovina (Đurić *et al*., 2015) showed that the amount of titratable acidity were in the range from 0.21 - 0.56% (Turkey) and 0.15 – 0.44% (B&H), which corresponds to our results. Variations are probably due to different climatic conditions and also cultivars.

Table 2. Titratable acidity (%) of evaluated pear cultivars (mean values and standard deviation)

Cultivars	Year 2012		Year 2013		Average	
	Mean	**Sd**	**Mean**	**Sd**	**Mean**	**Sd**
‘Ahmetova’	0.18^{cd}	0.019	0.35^{a}	0.019	0.26^{bc}	0.093
‘Budaljaca’	0.11^{d}	0.019	0.09^{d}	0.019	0.10^{e}	0.021
‘Dolokrahan’	0.23^{c}	0.011	0.12^{d}	0.019	0.18^{d}	0.063
‘Président Drouard’	0.17^{cd}	0.028	0.29^{b}	0.019	0.23^{c}	0.069
‘Hambarka’	0.37^{b}	0.018	0.19^{c}	0.019	0.28^{b}	0.102
‘Kacmorka’	0.24^{c}	0.027	0.20^{c}	0.001	0.22^{cd}	0.028
‘Krakaca’	0.73^{a}	0.030	0.29^{b}	0.019	0.51^{a}	0.243
‘Ljeskovaca’	0.29^{bc}	0.019	0.22^{c}	0.009	0.23^{c}	0.064
‘Sarajka’	0.22^{c}	0.070	0.22^{c}	0.009	0.22^{cd}	0.045
‘Takisa’	0.09^{d}	0.012	0.10^{d}	0.004	0.09^{e}	0.009
Average	0.26^{x}	0.179	0.21^{y}	0.085	0.23	0.142

a-e - different letters indicate statistically significant differences between cultivars at $p \leq 0.05$
x, y - indicate statistically significant differences between years at $p \leq 0.05$

The index of sweetness (Table 3) of evaluated pear cultivars varied from 49.21 (‘Krakaca’) to 192.68 (‘Takisa’). It was noteworthy that cultivar ‘Takisa’ had high soluble solids content but the lowest titratable acidity, and it is an appropriate cultivar for eating fresh. The index of sweetness (SSC/TA ratio) explains the sweet and sour balance in pears. For the preferred sweet and sour balance to occur, a relatively high ratio is required (Chen *et al*., 2007). Generally, a high acidity would result in a low acceptability (Boylson *et al*., 1994), but if the genotypes also have high SSC and a sweet taste, such as in the case of ‘Dolokrahan’ and ‘Sarajka’, it results in a relatively high SSC/TA ratio which is deemed more favourable.

Table 3. Index of sweetness of evaluated pear cultivars (mean values and standard deviation)

Cultivars	Year 2012		Year 2013		Average	
	Mean	**Sd**	**Mean**	**Sd**	**Mean**	**Sd**
'Ahmetova'	85.77[a]	9.72	52.97[b]	2.88	69.37[cd]	19.07
'Budaljaca'	153.12[a]	24.93	184.25[a]	43.38	168.68[a]	35.94
'Dolokrahan'	94.85[a]	5.97	175.87[b]	23.55	135.36[b]	46.96
'Président Drouard'	99.03[a]	15.64	66.10[b]	4.48	82.57[c]	20.76
'Hambarka'	56.35[de]	2.80	85.11[b]	10.33	70.73[cd]	17.14
'Kacmorka'	71.60[cd]	7.39	74.96[b]	0.57	73.27[cd]	5.04
'Krakaca'	28.53[e]	1.31	69.89[b]	4.44	49.21[d]	22.84
'Ljeskovaca'	62.33[cde]	4.84	80.76[b]	7.52	71.55[cd]	11.57
'Sarajka'	77.94[cd]	21.44	77.94	21.44	76.77[cd]	13.83
'Takisa'	196.91[a]	24.04	188.45[a]	11.35	192.68[a]	17.44
Average	92.64[y]	48.83	105.39[x]	54.14	99.02	51.51

a-e - different letters indicate statistically significant differences between cultivars at $p \leq 0.05$
x, y - indicate statistically significant differences between years at $p \leq 0.05$

Considering the average content of phenols (Table 4), the highest amount was measured in the cultivar 'Dolokrahan' (6808.44 mg GAE/kg of fresh fruit), while the smallest amount of phenols was observed in cultivar 'Ahmetova' (939.25 mg GAE/kg of fresh fruit). Related to the content of total phenols, statistically significant differences were achieved between cultivars and years. The content of secondary metabolites depends on the degree of ripeness, climatic conditions and other environmental impacts, species, variety and applied technological measures (Milošević *et al*., 2012). Cultivars 'Dolokrahan', 'Budaljaca' and 'Krakaca' have a very high content of phenolic compounds (6808.44; 4291.65 and 3424 mg GAE/kg of fresh fruit, respectively), particularly in comparison with literature data for pear varieties from Turkey and Chile (Galvic-Sanches *et al*., 2003; Ozturk *et al*., 2009), where the total phenol content does not exceed (500 mg GAE/kg and 2000 mg GAE/kg, respectively). This certainly supports the fact that indigenous varieties produce a greater amount of compounds that protect them against free radicals and pests by adapting to environmental conditions and growing without technological protection measures (Veberic *et al*., 2005).

Extremely high phenolic content in the cultivars 'Dolokrahan', 'Budaljaca' and 'Krakaca' is definitely a recommendation for their inclusion in the crossing programme.

Table 4. Total phenol content (mg GAE/kg of FW) of evaluated pear cultivars (mean values and standard deviation)

Cultivars	Year 2012		Year 2013		Average	
	Mean	**Sd**	**Mean**	**Sd**	**Mean**	**Sd**
'Ahmetova'	1093.79[c]	41.15	784.71[cd]	86.63	939.25[c]	179.83
'Budaljaca'	4562.04[b]	1002.89	4021.26[a]	446.86	4291.65[b]	754.93
'Dolokrahan'	10888.31[a]	13814.37	2728.58[b]	159.25	6808.44[a]	981.42
'Président Drouard'	2227.00[b]	269.98	882.44[cd]	133.85	1554.72[b]	760.70
'Hambarka'	3503.75[b]	538.36	670.48[cd]	8.10	2087.11[b]	1588.76
'Kacmorka'	2245.13[b]	106.90	1113.00[c]	40.16	1679.06[b]	624.29
'Krakaca'	5782.47[b]	326.24	1066.52[c]	63.83	3424.49[b]	2591.57
'Ljeskovaca'	3166.69[b]	269.73	1125.22[c]	71.32	2145.95[b]	1131.99
'Sarajka'	3255.24[b]	293.79	590.80[d]	64.26	1923.02[b]	1471.71
'Takisa'	2375.61[b]	222.50	880.07[cd]	16.49	1627.84[b]	831.21
Average	3910.00[x]	4526.01	1386.30[y]	1075.63	2648.15	3500.96

a-e - different letters indicate statistically significant differences between cultivars at $p \leq 0.05$
x, y - indicate statistically significant differences between years at $p \leq 0.05$

In order to overview the data for similarities and dissimilarities, PCA was applied on the calculated descriptors of studied compounds and resulted in a two-component model that explained 88.62% of the total variance (Table 5). PC1 and PC2, respectively, accounted for 61.27% and 27.35% of the total variability.

Table 5. Correlation coefficients between soluble solids content, titratable acidity, index of sweetness and total phenol content for 10 pear cultivars

Traits	Index of sweetness	SSC	TA	Total Phenols
Index of sweetness		0.0498	**-0.7849****	0.3521
SSC	0.0498		0.3753	**0.6697***
TA	**-0.7849****	0.3753		-0.0780
Total Phenols	0.3521	**0.6697***	-0.0780	

In this study high correlation coefficients with significant differences were achieved between the total soluble solids and total amount of phenol compounds (r=+0.67), as well as between the titratable acidity and index of sweetness (r=-0.78).

Table 6. Results of Principal Components Analysis related to biochemical composition of autochthonous pear cultivars

Component Number	Eigenvalue	Percent of Variance	Cumulative Percentage
1	2.4507	61.268	61.268
2	1.09406	27.352	88.619
3	0.419443	10.486	99.105
4	0.0357942	0.895	100.000

The principal components analysis distinguished two principal components which together covered 88.62% of the variations (Table 6). The principal component 1

(61.27% of the variations) included the highest impacts related to the total phenol content and index of sweetness in positive direction, and titratable acidity in opposite direction. The principal component 2 (27.35% variations) mostly comprised variations under the influence of soluble solids content. Out of the tested cultivars, two main groups were distinctive. The first group comprised ‘Dolokrahan’, ‘Takisa’ and ‘Budaljaca’ characterized by high amount of total phenol content and index of sweetness. The second group comprised ‘Ahmetova’, ‘President Drouard’ and ‘Krakaca’ characterized by high amount of titratable acidity.

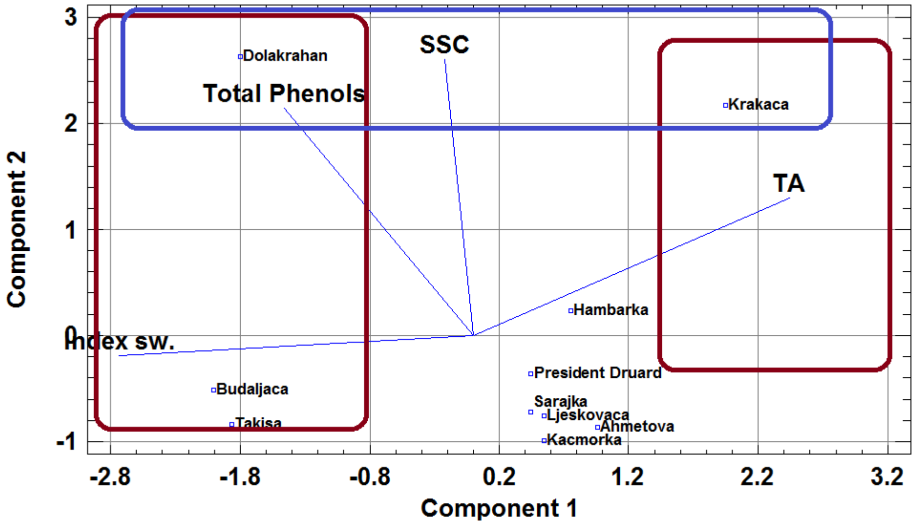


Figure. 1. Principal components analyses of studied pear cultivars with vectors of measured biochemical characteristics

CONCLUSION

The pear cultivars as well as growing season had considerable influences on all observed fruit characteristics. Based on the statistical analysis the autochthonous cultivars ‘Dolokrahan’ and ‘Krakaca’ had the greatest mean value of soluble solids content. The cultivar ‘Krakaca’ had the greatest value of titratable acidity, but lower value of sweetness index. The cultivars ‘Takisa’ and ‘Budaljaca’ achieved the greatest index of sweetness. Regarding the total amount of phenol compounds the cultivar ‘Dolokrahan’ reached the greatest average content of phenol compounds. The results of this study indicate diversity of autochthonous pear cultivars in Bosnia and Herzegovina related to biochemical composition of fruits. According to our results the cultivar ‘Dolokrahan’ has the greatest amount of phenols as well as good inedex of sweetness and soluble solids content. This cultivar could be recommended for fresh concumption and for manufacturing of jams and marmalades. Cultivars ‘Takisa’ and ‘Budaljaca’ are achieved the best index of sweetness and could be also use as good raw material in manufacturing of jams and marmalades. The pear cultivar 'Krakaca' could be used for juice production because their richness in soluble solids content and titratable acidity. Standard commercial cultivar 'President Druard' was not emphasized either at one of the analyzed parameters of fruit quality.

REFERENCES

Bostan, Z. S. (2009): Pomological traits of local apple and pear cultivars and types grown in Trabzon province (Eastern Black Sea Region of Turkey). Acta Horticulturae (ISHS), 825: 293–298.

Boylson, T. D., Kupferman, E. M., Foss, J. D., Buering, C. (1994): Sensory quality of 'Gala' apples as influenced by controlled and regular atmosphere storage. J. Food Quality, 17:477-494.

Chen, J., Wang, Z., Wu, J., Wang, Q., Hu, X. (2007): Chemical compositional characterization of eight pear cultivars grown in China. Food Chemistry, 104:268-275.

Dewanto, V., Wu, X., Adom, K. K., Liu, R. H. (2002): Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J. Agric. Food Chem. 50, 3010–3014.

Đurić, G., Mićić, N., Salkić, B. (2014): Evaluation of pear (*Pyrus communis* L.) germplasm collected in Bosnia and Herzegovina using some pomological and ecophysiological characteristics. Acta Horticulturae, 1032: 105−115.

Đurić, G., Tomić, L., Mićić, N., Cvetković, M., Radoš, Lj., Pašalić, B. (2009): Fruit genetic resources in Republika Srpska. Acta Agriculturae Serbica XIV, 28: 31−40.

Đurić, G., Žabić, M., Rodić, M., Stanivuković, S., Bosančić, B., Pašalić, B. (2015): Biochemical and pomological assessment of European pear accessions from Bosnia and Herzegovina. Hort. Sci. (Prague), 42, 4: 176–184

Gasi, F., Kurtovic, M., Kalamujic, B., Pojskic, N., Grahic, J., Kaiser, C., Meland, M. (2013): Assessment of European pear (*Pyrus communis* L.) genetic resources in Bosnia and Herzegovina using microsatellite markers. Scientia Horticulturae, 157: 74−83.

Jakopic, J., Stampar, F., Veberic, R. (2009): The influence of exposure to light on the phenolic content of "Fuji" apple. Scientia Horticulturae, 123: 234−239.

Kellerhals, M., Bertschinger, L., Gessler, C. (2004). Use of genetic resources in apple breeding and for sustainable fruit production. Journal of Fruit and Ornamental Plant Research 12:53-62.

Milošević, T., Milošević, N., Glišić, I., Mladenović, J. (2012): Fruit quality attributes of blackberry grown under limited environmental conditions. Plant, Soil and Environment, 58: 322−327.

Ozturk, I., Ercisli, S., Kalkan, F., Bunyamin, D. (2009): Some chemical and physico-mechanical properties of pear cultivars. African Journal of Biotechnology, 8: 687−693.

Percival, I. C. (1998): Quantum transfer functions, weak nonlocality and relativity. Physics Letters A, 244: 495–501.

Rufino, M. S. M., Alves, R. E., Fernandes, F. A. N., Brito, E. S. (2011): Free radical scavenging behaviour of ten exotic tropical fruits extracts. Food Research International, 44: 2072−2075.

Selamovska, A., Milevska, M. E., Najdenovska, O. (2013): Genetic resources of traditional pear varieties in the region of Skopje. Section of Natural, Mathematical and Biotechnical Sciences, 34: 93–100.

Selamovska, A., Milevska, M. E., Najdenovska, O., Dimovska, D. (2014): Traditional pear varieties in the west region of Republic of Macedonia. Acta Agriculturae Serbica, 19: 47–60.

Veberic, R., Trobec, M., Herbinger, K., Hofer, M., Grill, D., Stampar, F. (2005): Phenolic compounds in some apple (*Malus domestica* Borkh.) cultivars of organic and integrated production. Journal of the Science of Food and Agriculture, 85: 1687–1694.

FIRST EXPERIENCE WITH SOME NEW FLAT PEACH AND NECTARINE CULTIVARS*

Metka Hudina[1]

Original scientific paper

Summary

15 new flat peach and nectarine cultivars were planted in March 2012 (yellow flesh flat peach cultivars: Plane Gem, Plane Top, Plane Sun, Plane Gold, Ornella, Oriane, Ordigan; white flesh flat peach cultivars: UFO 3, UFO 4, Platifirst, Early Sandwich, Platicarpa Bianca, Platibell, Platifun; yellow flesh flat nectarine cultivar: Platimoon) and compared to standard peach cultivars Norman and Veteran. Trees were grafted on GF 677 rootstock, growing spindle, at planting distance of 4 x 2 m. For each cultivar 12 plants (3 x 4) were planted. Flowering and harvest date, number of fruits/tree, yield per tree and hectare, fruit dimension and organoleptic grade were observed. In 2013 we already had the first crop. The highest yield had cvs. UFO 4 (2.4 t/ha) and UFO 3 (2.1 t/ha). Flat peach cultivar Oriane and standard peach cultivars Veteran and Norman didn't have yield in 2013 yet. The highest yield in 2014 had cvs. UFO 4 and Plane Sun (18.7 t/ha and 15.9 t/ha), folowed by UFO 3 (14.2 t/ha) and Plane Gem (13.7 t/ha). Standard peach cultivars Veteran and Norman also didn't have yield in 2014. The highest yield in 2015 had standard cultivar Veteran (20.1 t/ha), followed by cvs. Plane Gold and Platicarpa Bianca (18.2 t/ha and 16.4 t/ha), UFO 3 (11.6 t/ha) and UFO 4 (10.8 t/ha). In 2016 the highest yield had standard cultivar Veteran (35.3 t/ha). The yield higher than 20 t/ha had cvs. Plane Gold (26.4 t/ha), Oriane (24.5 t/ha) and Plane Top (22.6 t/ha); less than 10 t/ha had cvs. Platifirst, Platifun and Platimoon; other cultivars had a yield of between 10 and 20 t/ha in 2016. The best value of the fruit (excellent) was given to cultivars UFO 4, Platicarpa Bianca, Platimoon, Platifun and Platibell.

Key words: *flat peach, flat nectarine, cultivar testing, yield, pomological characteristics*

INTRODUCTION

Peaches are the third most important deciduous tree fruits worldwide and have a long history. They originated in China and, over the centuries, spread out to the rest of Asia and Western countries, and then crossed the Atlantic to the Americas. New peach and

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1]University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Jamnikarjeva 101, 1000 Ljubljana, Slovenia, E-mail: metka.hudina@bf.uni-lj.si

nectarine cultivars are being developed with the aim to obtain fruits with improved flesh color, melting flesh, good size, firmness, and enhanced flavor (Cantín *et al*., 2010). The major objective of the breeding programs is to develop new genotypes that express the flavor before the fruit becomes too soft (Iglesias and Echevarría, 2009).

A flat peach is a natural genetic mutation. The shape of the fruit is naturally flat rather than globose (spherical or round). They are known by many other names, including doughnut peach, paraguayo peach, pan tao peach, saucer peach, flat peach, belly-up peach, UFO peach, Chinese flat peach, hat peach, anjeer peach, custard peach, pumpkin peach, squashed peach or pita peach. They have yellow or white flesh. The flesh texture may be melting or nonmelting. The pit inside of flat peach also has a different shape than a traditional peach pit; the pit is nearly spherical (Clemson …, 2017). The flavor of flat peaches is unique because of their aromas, intense sweetness, and very good to excellent taste when ripe (Iglesias and Carbó, 2009). The most important progress in breeding in flat peaches and nectarines has been achieved mainly in increasing fruit size, fruit color, and adding a wide range of maturity. Breeders have obtained flat shape fruits containing a dominant flat allele, with increasing popularity (Martínez-Madrid *et al*., 2000).

With testing and introduction of foreign cultivars, in Slovenia, we enrich the assortment of fruit species, which consists almost exclusively of foreign cultivars. We want to determine the suitability of new cultivars for our soil and climatic conditions. They must be characterized by regular and abundant yield, yielding quality fruit, resistance to environmental stresses, pests and diseases. The characteristics must fulfil the requirements of the market (Layne and Bassi, 2008). A new cultivar must have at least one important characteristic better in comparison with the standard one. If it fits the requirements, it is included in the fruit variety list and in fruit production. In the evaluation of new flat peach and nectarine cultivars, information on yield, inner fruit quality and external characteristics are crucial. The useful methods in the evaluation of peach and nectarine fruit quality are sensory ratings of fruit by panelists and physical measurements of fruit properties (Bassi and Selli, 1990; Brovelli *et al*., 1999; Versari *et al*., 2002; Aubert *et al*., 2003).

The aim of this research was to determine suitable cultivars for flat peach and nectarine grown in the ecological and pedological conditions of Slovenia.

MATERIALS AND METHODS

The following 15 new flat peach and nectarine cultivars were planted in March 2012: yellow flesh flat peach cultivars: Plane Gem, Plane Top, Plane Sun, Plane Gold, Ornella, Oriane, Ordigan; white flesh flat peach cultivars: UFO 3, UFO 4, Platifirst, Early Sandwich, Platicarpa Bianca, Platibell, Platifun; and yellow flesh flat nectarine cultivar: Platimoon. This flat peach and nectarine cultivars were compared to standard peach cultivars Norman and Veteran. Trees were grafted on GF 677 rootstock. The trees were grown according to the spindle training system at planting distance of 4 x 2 m. For each cultivar 12 plants (3 x 4) of flat peaches and nectarine were planted. Observation started in 2013, 1 year after planting, because some cultivars already had their first yield. Fruit of each cultivar were harvested at commercial maturity. The orchard was managed according to standard commercial practice for integrated fruit production (i.e., pruning, spraying, irrigation, etc.) (Ministry of Agriculture, Forestry and Food, 2016).

The phenological properties (beginning and end of blooming) according to Winter (2002) and dates of harvest were monitored from 2013 to 2016. Physical characteristics such as number of fruit and yield per tree and per ha, and fruit weight were determined. Ten panelists performed the sensory evaluation. The same panellists evaluated the attributes of fruits in all cultivars. Each of them independently estimated ten fruits of typical size, appearance and similar ripeness per cultivar. The evaluated samples were not named until the end of the evaluation. The evaluations were performed at room temperature (24 °C). The harvested fruits were stored (at 4 °C) up to 36 h before sensory evaluation.

RESULTS AND DISCUSSION

Flat peaches and nectarines blossomed from the 3th March to 19th April in 2013 to 2016 (Fig. 1). The earliest in beginning of flowering was cultivar Early Sandwich, followed by Platifirst, Platibell and Platifun, and the latest was standard cultivar Veteran.

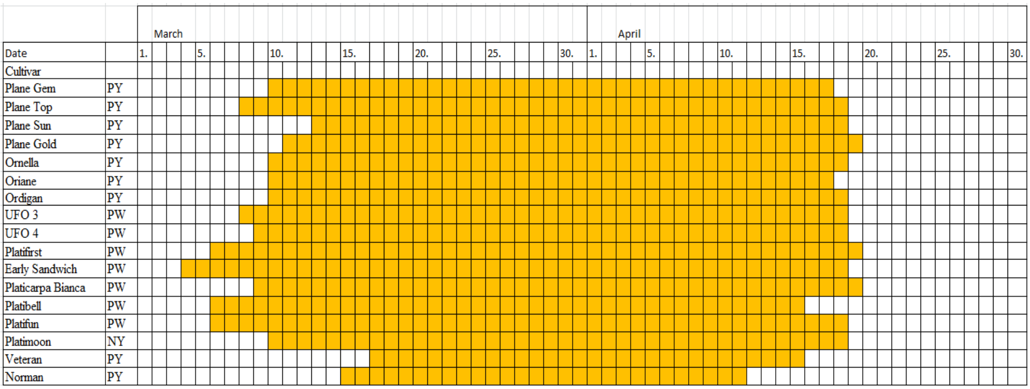


Fig. 1. Flowering date (beginning and end) of flat peach and nectarine cultivars; 2013-2016 (PY-yellow flesh peach, PW-white flesh peach, NY-yellow flesh nectarine).

According to the ripening period, the earliest cultivar was Early Sandwich, which ripped from 29th May to 18th June in our experimental years (2013-2016). The next after the ripening period is the UFO 3 cultivar, which ripped from 16th June to 3th July. UFO 4 cultivar was ripening from 7th June to 9th July, Platifirst from 30th to 9th July, Platicarpa Bianca fom 30th June to 9th July, Plane Gem from 19th to 26th July, Plane Sun and Platibell from 7th July to 2nd August, Platifun from 17th July to 2nd August, Platimoon from 25th July to 19th August, Ornella from 28th July to 19th August, Plane Gold from 25th July to 25th August, Oriane from 1st to 26th August and Ordigan from 28th July to 26th August. The last to ripen was Plane Top, which ripped from 1st to 29th August. Standard cultivars Norman and Veteran ripped from 1st to 9th August and from 10th to 20th August, respectively.

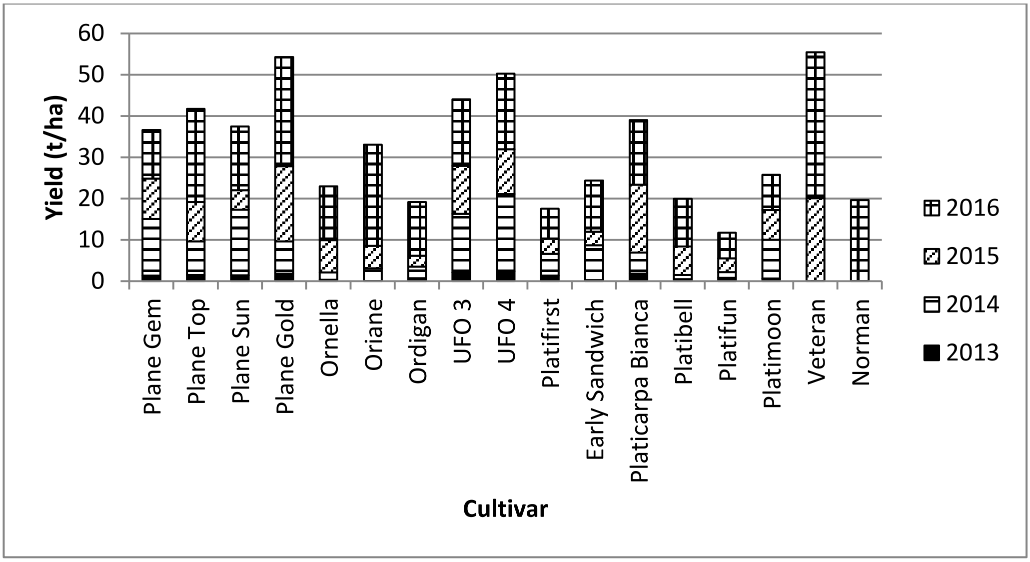


Fig. 2. Cumulative yield/ha and yield of flat peach and nectarine cultivars in 2013, 2014, 2015 and 2016.

In 2013, the flat peach and nectarine trees had their first yield. The Ornella, Oriane and Early Sandwich cultivars had no fruit in 2013 (Fig. 2). Standard cultivar Veteran had first yield in 2015 and Norman only in 2016. The yield/ha differed between years and cultivars. Higher cumulative yield/ha than 40 t was observed in Plane Top, Plane Gold, UFO 3, UFO 4 cultivars and standard cultivar Veteran. According to literature cv. Plane Gold is characterized by its high quality fruit and regular yield (Provedo, 2017). Cultivar Plane Top is characterized by great and regular yield (Provedo, 2017). Also at cultivar UFO 3 yield is great and regular (Zanzi and Zanzi, 2013).

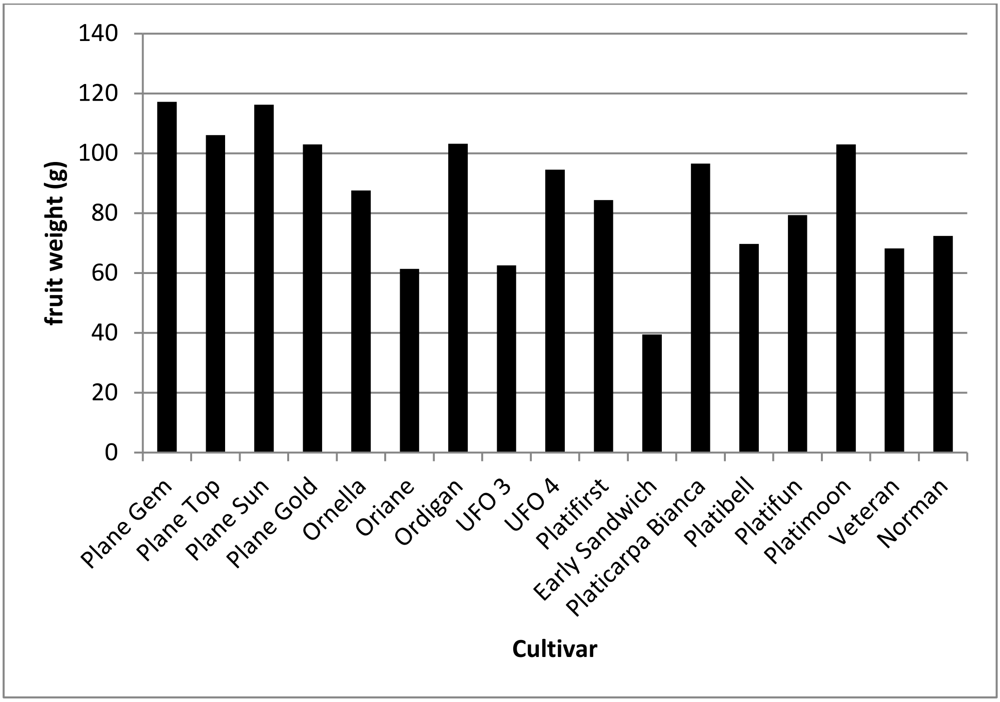


Fig. 3. Average fruit weight of flat peach and nectarine cultivars; 2013-2016

Average fruit weight more than 100 g had cultivars Plane Gem, Plane Sun, Plane Top, Plane Gold, Ordigan and Platimoon (Fig. 3). The smallest fruit had cultivar Early Sandwich, only 39,5 g in average.

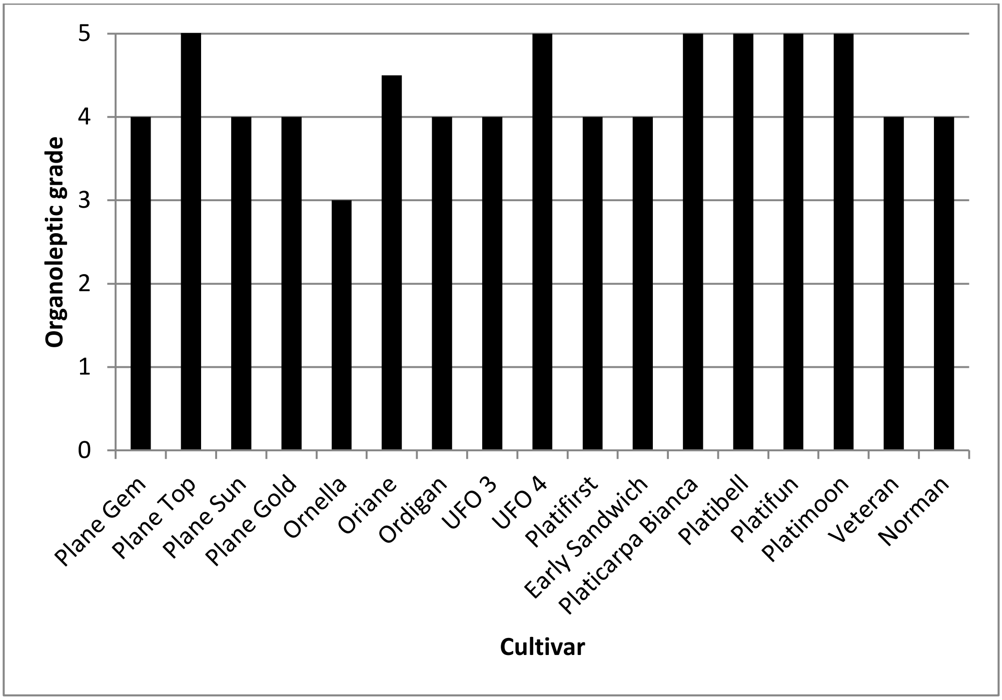


Fig. 4. Organoleptic grade of flat peach and nectarine cultivars; 2013-2016.

The results of a previous study (Colaric *et al*., 2005) indicate that sensory evaluation provides a good tool for rapid assessment of peach and nectarine fruit quality. Excellent sensory evaluation was observed for the UFO 4, Platicarpa Bianca, Platibell, Platifun and Platimoon cultivars, which was evaluated as excellent in all four years. Sensory evaluation for Ornella cultivar was good (Fig. 4). Fruit of Platicarpa Bianca have sweet taste with low acidity, the flesh is aromatic (Peschi, 2017). Cultivar Platibell fruit have subacid-sweet flavour, white melting flesh, fine flesh texture and are very good. Fruit of cultivar Platifun have very pleasant sweet subacid flavour, melting white flesh and are very firm. Cultivar Platimoon fruit have sweet flavour with intense aroma; flesh is melting and crispy. UFO 4 is cultivar with high and consistent yield of large fruit with excellent flavour (Zanzi and Zanzi, 2013).

CONCLUSIONS

Cumulative yield 2013-2016 more than 44 t/ha achieved cultivars Plane Gold, UFO 4 and UFO 3, which had organoleptic grade very good, excellent and very good, respectively. Cultivar Plane Gold had fruit of 103 g and had four year cumulative yield 54 t/ha. Fruit weight of cultivar UFO 3 was 63 g and cumulative yield 44 t/ha. Cultivar UFO 4 had fruit of 94 g and had four year cumulative yield 50 t/ha. According to the first four years of observation we can recommend for further growing flat peach cultivars UFO 4, UFO 3 and Plane Gold.

ACKNOWLEDGEMENTS

This study was a part of the Special testing of new varieties of fruit plants. The authors thank the Ministry of Agriculture, Forestry and Food and the Slovenian Research Agency for financial support. The author is grateful to Fruit-growing centre Bilje for providing an experimental site and maintaining the trial in optimum conditions throughout the study.

REFERENCES

Aubert, C., Gunata, Z., Ambid, C., Baumes, R. (2003): Changes in physicochemical characteristics and volatile constituents of yellow- and white-fleshed nectarines during maturation and artificial ripening. J. Agric. Food Chem., 51: 3083-3091.

Bassi, D., Selli, R. (1990): Evaluation of fruit quality in peach and apricot. Adv. Hort. Sci. 4: 107-112.

Brovelli, E. A., Brecht, J. K., Sherman, W. B., Sims, C. A., Harrison, J. M. (1999): Sensory and compositional attributes of melting and non-melting-flesh peaches for the fresh market. J. Sci. Food Agric., 79: 707-712.

Cantín, C. M., Gogorcena. Y., Moreno. M. A. (2010): Phenotipic diversity and relationship of fruit quality traits in peach and nectarine [*Prunus persica* (L.) Batsch] breeding progenies. Euphytica, 171: 211-216.

Clemson cooperative extension. Frequently Asked Questions. 2017. http://www.clemson.edu/extension/peach/faq/ [2017 Oct. 27].

Colaric, M., Veberic, R., Stampar, F., Hudina, M. (2005): Evaluation of peach and nectarine fruit quality and correlations between sensory and chemical attributes. J. Sci. Food Agric., 85: 2611-2616.

Iglesias, I., Carbó, J. (2009): Melocotón y nectarina plana: las variedades de mayor interés. Ed.: Institut de Recerca i Tenologia Agroalimentaries (IRTA). Barcelona (Spain). 134 pp.

Iglesias, I., Echeverría, G. (2009): Differential effect of cultivar and harvest date on nectarine colour, quality and consumer acceptance. Sci. Hortic., 120: 41-50.

Layne, D., Bassi, D. (2008): The peach. Botany, production and uses. Wallingford, CABI, 615 pp.

Martínez-Madrid, M. C., Serrano, M., Pretel, M. T., Martínez-Reina, G., Romojaro, F. (2000): The ripening of *Prunus persica* fruits with a dominant flat allele. Food Sci. Technol. Int., 6: 399-405.

Ministry of Agriculture, Forestry and Food. (2016): Tehnološka navodila za integrirano pridelavo sadja.

http://www.mkgp.gov.si/si/delovna_podrocja/kmetijstvo/integrirana_pridelava/tehnoloska_navodila/ [2017 Oct. 27].

Peschi. (2017): Platicarpa Bianca. http://www.fruttiantichi.biz/mpc/52/mpcn/Peschi/pc/307/pcn/platicarpa bianca/lty/B/prodotti.aspx [2017 Oct. 27].

Provedo. (2017): New varieties peach, nectarine, flat peach and platerina. http://www.extremebyprovedo.com/varieties/yellow-flesh-flat-peach?phpMyAdmin=WIk3Si8MyuiScZoyoYVf9M2mbzf [2017 Oct. 27].

Versari, A., Castellari, M., Parpinello, G. P., Riponi, C., Galassi, S. (2002): Characterisation of peach juices obtained from cultivars Redhaven, Suncrest and Maria Marta grown in Italy. Food Chem., 76: 181-185.

Winter, F. (2002): Lucas' Anleitung zum Obstbau. Verlag Eugen Ulmer, Stuttgart, Germany, 448 pp.

Zanzi, V., Zanzi, C. (2013): Peach. http://www.vivaizanzi.it/UserFiles/File/brochure/011Pesco_GB.pdf [2017 Oct. 27].

PHENOLOGICAL CHARACTERISTICS OF NEWLY INTRODUCED VARIETIES OF NECTARINES (“SUN GRAND“,“CALDESI 2000“ AND “VENUS“) IN HERZEGOVINA*

FENOLOŠKE KARAKTERISTIKE NOVOINTRODUKOVANIH KULTIVARA NEKTARINE (“SUN GRAND“,“CALDESI 2000“ I “VENUS“) U HERCEGOVINI

Jasna Hasanbegović[1]**, Jasmina Aliman[1], Semina Hadžiabulić[1], Ahmed Džubur[1], Alma Leto[1], Azra Skender[2]

Original scientific paper

Summary

The paper presents the results from a two-year long research on phenological characteristics of three newly introduced varieties of nectarines (“Sun Grand”, “Caldesi 2000” and “Venus”) in ecological conditions of Herzegovina (Mostar region). The research was conducted during 2014 and 2015 and included the identification of flowers types, blossoming phenophases monitoring, energy of blossoming and maturation time. The rootstock for all genotypes is GF 677. Rose-shape flower type was recorded at nectarine varieties “Sun Grand“ and “Venus“, while for the variety “Caldesi 2000” bell-shape flower type was recorded. The earliest beginning of blossoming in both research years was recorded for “Sun Grand” variety, while the latest blossoming was recorded for “Venus” variety. Beginning of blossoming, as well as energy of blossoming of nectarine varieties, varied in dependence to the year, so all the nectarine varieties had earlier beginning of blossoming in 2014 with the longer blossoming duration, comparing to 2015. The earliest fruit setting and fruit maturation was recorded for variety “Sun Grand“, while the latest was recorded for the variety of “Venus“. The shortest period from fruit formating to the maturation was recorded for the variety of “Sun Grand“ (56,6 days), while the longest period was observed for the variety of “Venus“ (76 days). When it comes to phenological characteristics, all the varieties showed the significant adaptability to agro-ecological conditions of Herzegovina and they may be recommended for commercial production.

Key words: *nectarine, newly introduced varieties, flower type, flowering phenophases*

---

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] University “Džemal Bijedić“ Mostar, Agromediterranean Faculty, Bosnia and Herzegovina
[2] University of Bihać, Biotechnical Faculty, Bosnia and Herzegovina
**Corresponding author: jasna.hasanbegovic@hotmail.com

Rezime

U radu su prikazani rezultati dvogodišnjeg istraživanja fenoloških karakteristika tri novointrodukovana kultivara nektarine ("Sun Grand", "Caldesi 2000" i "Venus") u ekološkim uslovima Hercegovine (područje Mostara). Istraživanje je sprovedeno tokom 2014. i 2015. godine i uključivalo je identifikaciju tipa cvijeta, fenofaze cvjetanje, energiju cvjetanja i sazrijevanja plodova. Podloga za sve genotipove je GF 677. Ružast tip cvijeta je zabilježen kod kultivara nektarine "Sun Grand" i "Venus", dok je za kultivar "Caldesi 2000" zabilježen ružast tip cvijeta. Najraniji početak cvjetanja u obe istraživačke godine zabilježen je kod kultivara "Sun Grand", a najkasniji početak cvjetanja zabilježen je kod kultivara "Venus". Početak cvjetanja, kao i energija cvjetanja kultivara nektarine, varirala je u zavisnosti od godine, tako da su svi kultivari nektarine ranije počeli cvjetati 2014., sa dužim trajanjem cvjetanja, u poređenju sa 2015. godinom. Najranije zametanje i sazrijevanje plodova zabilježeno je kod kultivara "Sun Grand", a najkasnije je zabilježeno kod kultivara "Venus". Najkraći period od zametanja plodova do sazrijevanja zabilježen je kod kultivara "Sun Grand" (56,6 dana), dok je najduži period zabilježen kod kultivara "Venus" (76 dana). Kada su u pitanju fenološke karakteristike, svi kultivari pokazali su značajnu prilagodljivost agroekološkim uslovima Hercegovine i mogu se preporučiti za komercijalnu proizvodnju.

Ključne riječi: *nektarine, novointrodukovani kultivari, tip cvijeta, fenofaze cvjetanja*

INTRODUCTION

Nectarine is a sub-tropical fruit treewhich requires irrigation, thinning and disease protection to be commercially viable (Naor *et al.* 2001). In recent years, increasing of nectarine production in Herzegovina includes new varietiers with different growth and fruiting characteristics, harvest date, more full white fruit colour and better eating quality than the existing old varieties.

A peach and a nectarine are very similar. Genetically, there is not much difference between the two. The main difference is that a peach has fuzz on its skin while a nectarine does not. Nectarines also tend to be smaller and more aromatic than peaches and have more red color on the fruit surface. Nectarine fruits may have white, yellow, or red flesh, just as peaches do. Their seeds cannot be distinguished from peach seeds.

Like other stonefruit, peaches and nectarines, both closely related Brady (1993), have a characteristic, lignified endocarp (pit or stone) that encloses the seed, a fleshy mesocarp and a thin exocarp (Lill *et al.* 1989).

The goal of this paper was to determine up to which extent the introduced varieties nectarines ("Sun Grand", "Caldesi 2000" and "Venus") have adapted to agro-ecological conditions in Herzegovina as well as do they meet the needs of commercial production by their physical-chemical characteristics.

The pace of nectarine expansion in our country is relatively slow and significantly lapses behind the ordinary peach. There are several reasons for this outcome: compared to varieties of ordinary peach, nectarine varieties are more susceptible to low temperatures, provide less yields, have smaller fruit, and are more perceptible to factors that cause diseases (especially asbestos and fruit rot). In addition, the nectarine assortment in our country is quite obsolete. A relatively small number of varieties, which mostly ripen in the period from the beginning of July to the middle of August, is present in the production (Mratinić *et al.*, 2003).

Being acquainted with the flow of inflorescence phenophase is significant for determining the assortment in a particular ecological area, given the climatic conditions of the area.

In addition to the hereditary basis, the beginning and duration of blooming is also influenced by the climate and orographic factors, depending on the year of the research, as well as the specific microclimatic characteristics of the site.

Of all climatic conditions, temperature and wind are the major factors, which can adversely affect blooming, intensity and duration of inflorescence, fertilization and fruit setting.

The duration of blossom is 8-10 days. If the relative humidity is lower and the temperature is higher during the inflorescence, then their opening is much faster and can be completed in a few hours.

Since nectarine blossoming is relatively long, fertilization is successful even in poor meteorological conditions. That is the reason why nectarines have a high percentage of fertilization, despite the fact that each flower has only one seed embryo.

Nectarine belongs to fruit species with earlier blossom, and the beginning of blossoming between different varieties varies from 7 (Pejkić, 1982) to 15 days (Niketić, 1956), while (Milošević, 1996) states that these differences may be less, so in agroecological conditions in Čačak, the beginning of blossoming is less than 5 days during some years.

MATERIAL AND METHODS

The analysis was performed in the ecological conditions of the middle flow of the Neretva River at Blagaj, in the production plant "Jaffa-komerc".

The research included monitoring of the dynamics and the energy of blossom of examined nectarine varieties during the two vegetation (2014-2015). The experiment was set by "randomized block design method" with 5 trees per variety - 25 trees in total. The plantation is in full fertility. Planting spacings are 4 x 2.5 m, and the breeding form is a modified slender spindle. The research was carried out on new nectarine varieties: "Sun Grand", "Caldesi 2000" and "Venus". The rootstock for all investigated genotypes is GF 677. In agriculturally and mentally high-intensity treatment (soil treatment, nutrition, protection) is carried out in farms. An agrotechnical and pomotechnical treatment of high intensity (soil treatment, nutrition, protection) is carried out in the plantations.

The beginning of blossom is defined as the date when 10-20% of flowers were opened on the trees, full blossom when 90% of the flowers were opened and the end of flowering (precipitation) when 90% of the petals fell off.
The blossom date was recorded for each nectarine variety. The average start date of flowering (E phase), full flow (C phase) and the end of flowering (G phase) were determined according to (Fleckinger, 1945), and (Slović, 1972).

RESULTS AND DISCUSSION

The type and the color of the flower

The results of the flower type and color of the examined nectarine varieties are presented in Table 1.
For the majority of nectarine varieties, two types of flowers are represented, and they are: rose-shape (Rose-shape type), which according to the IBPGR (1984) descriptor is marked with (1) and bell-shape, marked with (2).

Table 1. The type and the color of flowers in nectarine varieties in the period 2014-2015

	Sun Grand	**Caldesi 2000**	**Venus**
Flower description	Rose-shape	Bell-shape	Rose-shape
Flower color	light pink	dark pink	light pink
Mark	1	2	1

The rosaceous type of flower - the mark (1) was recorded in varieties of nectarine "Sun Grand" and "Venus" that were the subject of this research. Variety "Caldesi 2000" is characterized by a bell-shaped flower (2).
The color of the flower in the examined varieties was pink in different shades of light to dark (Table 1).

Nectarine blossomig phenophases

The results of the monitoring of the flow rate of the blossom phenophase of the nectarine varieties are shown in Table 2 and phenograms 1 and 2 (Charts 1 and 2).

Table 2. The blossom phenophases of examined nectarine varieties in the period 2014-2015

	Sun Grand			**Caldesi 2000**			**Venus**		
	2014	**2015**	**Average**	**2014**	**2015**	**Average**	**2014**	**2015**	**Average**
Beginning	1st of April	14th of March	22nd of March	4th of April	21st of March	28th of March	8th of April	23rd of March	31st of March
Full	10th of April	21st of March	31st of March	14th of April	28th of March	5th of April	17th of April	1st of April	9th of April
End	12th of April	24th of March	30th of March	17th of April	2nd of April	9th of April	22nd of April	6th of April	14th of April

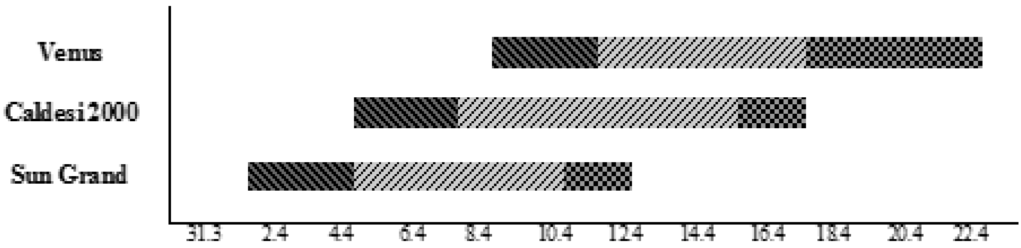


Chart 1. The blossom phenogram of examined nectarine varieties in 2014

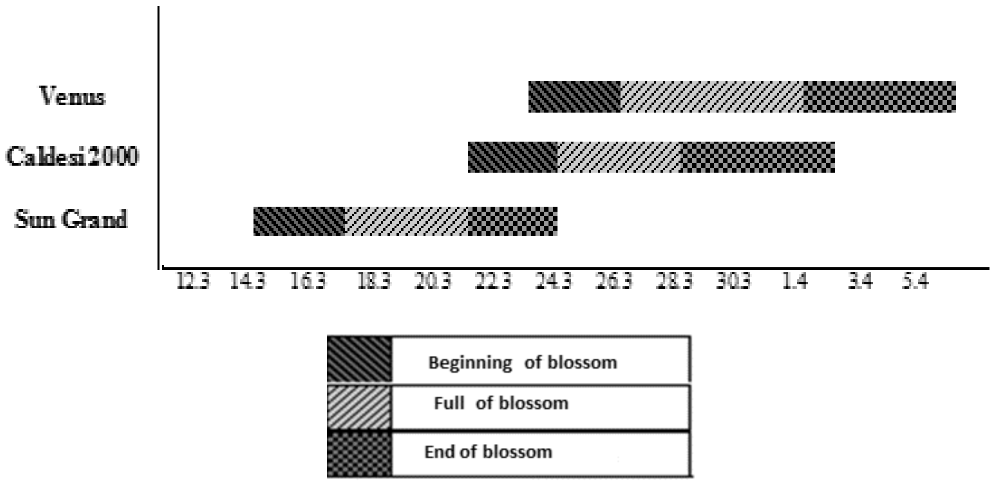


Chart 2. The blossom phenogram of examined nectarine varieties in 2015

On the basis of the results shown in Table 2 and blossomin phases 1 and 2, it can be concluded that there are no significant differences in the start time of blossoming between individual varieties in the investigated period which is 8 days. The earliest blossoming in the investigated period was recorded at variety "Sun Grand" (March 14th 2015), and the latest at the variety "Venus" (April 8th 2014).
In all varieties, in comparison with 2014, the earlier beginning of blossoming was recorded in 2015 (12-17 days earlier), which was the result of significantly more favorable weather conditions that marked the period before and during the blossoming in 2015.

According to (Zec, 2009), in the research conducted in the period 2006-2008, the variety "Venus" began to blossom on March 18th and finished on April 25th with the duration of blossom for 12 days.

The influence of agroecological conditions in the examined region contributed to the fact that all examined varieties were grouped early to medium blossoming varieties (Bellini *et al.*, 1984). The state of the agroecological conditions at the investigated site, namely the temperature and precipitation, influenced the time of blossoming of the studied varieties.

The blossoming phenophase mostly depends on biological, climatic and orographic factors, as well as on the age of the variety, the type of rootstock, the way of cutting and sunlight exposure (Bulatović, 1992). In regards to the blossoming time, the examined nectarine varieties are in the interval from early to medium blossom varieties. The earliest average blossom was recorded at "Sun Grand" variety, and the latest by "Venus" variety.
The results of the observation of "Venus" variety blossoming are similar to the results of Özkan and Özdil, 2012, however, different from the results obtained by Prenkić and Odalović, 2009, who cite a much earlier beginning of the blossoming of varieties "Venus" and "Caldesi 2000".
The observation of nectarine varieties blossoming is similar to that of Milošević, 1996, Glišić *et al.* 2008, Vujanić-Varga *et al.* 1998.

The results of the study (Odalović *et al.* 2003) *in* the agroecological conditions of the Ćemovsko field show that the beginning of the nectarine blossoming phenophase was at the end of February, while (Prenkić and Odalović, 2007) recorded the beginning of blossoming in the same region as the first half of March.
This is a very important varietal characteristic, because during some years even a day or two earlier blossoming can have severe consequences for fertility and yield at sites exposed to the effects of late spring frosts. In addition, this sequence of flowering confirms the allegations (Baldini, Scaramuzzi, 1982) that in nectarine there is no connection between the time of flowering and the time of maturation, because the varieties that blossom earlier do not have to have earlier maturation, as is the case with other fruit species.

The results for the blossom energy of examined nectarine varieties are shown in Table 3.

Table 3. The blossom flow of examined nectarine varieties in the period 2014-2015

	Sun Grand	**Caldesi 2000**	**Venus**	**Average**
2014	**12**	13	14	13
2015	10	12	**15**	12,33
Average	11	12,5	14,5	

By analyzing the data on the duration of blossom from Table 3. it can be noted that in 2014, the varieties of "Sun Grand" and "Caldesi 2000" recorded a longer blossom period, whereas the variety "Venus" blossom period was shorter than in 2015. The shortest blossom in the study period was recorded in the "Sun Grand" variety in 2015, and the longest with the "Venus" variety in the same year.

An increased dynamism of blossom in 2015 was conditioned by elevated average daily air temperatures, which occurred in the blossom aftermath, while in 2014 they experienced a decline in that period.

Prenkić and Odalović (2009) state that for the period 2004-2006 the variety "Caldesi 2000" has been blooming for 12 days on average, whereas the "Venus" variety was blossom for 13 days, which is approximately the data obtained in this study.

Time of maturation of examined nectarine varieties

The results of the observation of the time of maturation of the examined nectarine varieties are presented in Table 5.

Table 5. Fruiting phenophases of examined nectarine varieties in the period 2014-2015

	Sun Grand			**Caldesi 2000**			**Venus**		
	2014	**2015**	**Average**	**2014**	**2015**	**Average**	**2014**	**2015**	**Average**
Fruit formation	29th of Apr.	15th of Apr.	22nd of Apr.	9th of May	17th of Apr.	28th of Apr.	15th of May	21st of Apr.	3rd of May
Straw	9th of May	5th of Jun.	23rd of May	20th of Jun.	20th of Jun.	20th of Jun.	15th of Jul.	29th of Jun.	22nd of Jun.
Fruit maturation	13th of Jun.	20th of Jun.	16th of Jun.	1st of Jul.	3rd of Jul.	2nd of Jul.	31st of Jul.	3rd of Avg.	2nd of Avg.

The earliest fruit formation and fruit maturation of fruits was recorded at "Sun Grand" variety in both research years. Variety "Venus" had the latest fruit formation and maturation of the fruit in both years of research, and this variety can be classified into varieties of late maturation time.

Variety "Caldesi 2000" is a variety of medium late maturation time. The maturation of this variety in both years 2014 and 2015 varied the least (two days of difference). The maturation time of most examined nectarine varieties coincide with the research to a large extent (Odalović, 2002).

The results of the research Prenkić and Odalović (2009) for the variety "Venus" coincide with the results of this research, while they deviate for the variety "Caldesi 2000".

Nicotra *et al.* (1994) and Forgle and Scorza (1982) state that higher amplitudes at maturation time were recorded in varieties of the earlier epoch of maturation than in varieties of medium late and late epochs of maturation in different agroecological conditions.

Fruit development period of examined nectarine varieties

The results of analyzing the period fruit development are shown in Table 6.

Table 6. Duration period of fruit development in the period 2014-2015

	Sun Grand	Caldesi 2000	Venus	Average
2014	46	54	78	59,3
2015	67	78	74	73
Average	56,5	66	76	

The shortest period of fruit development was recorded at Sun Grand variety in 2014 (46 days), and the longest period had the variety "Venus" in that year (78 days).
On the basis of this data, it can be concluded that the "Sun Grand" variety belongs to the category of early maturation varieties, and the variety "Venus" is a category of late maturation varieties.
Variety "Caldesi 2000" is a medium late maturation variety, compared to the other two varieties (Table 6).

CONCLUSIONS

On the basis of examined phenological observations of examined nectarine varieties ("Sun Grand", "Caldesi 2000" and "Venus") at the site of Mostar, the following conclusions can be made:
Varieties "Sun Grand" and "Venus" have a rose-shape type of flower, light pink, while a bell-shaped flower with a dark pink color is recorded in "Caldesi 2000" variety.
Early blossoming, fruit formation and maturation in the examined period 2014-2015 was recorded at "Sun Grand varieties, and the latest ones were examined at variety "Venus". The shortest blossoming in the study period was recorded in the "Sun Grand" variety in 2015, and the longest with the Venus variety in the same year. In the end, it can be concluded that the examined varieties of nectarine "Caldesi 2000" and "Venus" have shown exceptional adaptability to the agro-ecological conditions of Herzegovina and with the application of adequate agro-technical, pragmatic measures and protection they can be economically justified, and these varieties are certainly recommended for further cultivation in this area.
It should be noted that extreme high temperatures in the final stage of maturation of the fruit of the variety "Venus" can cause fruit fracture, as happened in 2015, but for these reasons it is necessary to intensively carry out continuous irrigation in this period.
In both research years the variety "Sun Grand" has not shown satisfactory results in terms of resistance to spring frosts, as well as the satisfactory size and quality of fruits. From this paper, it can be concluded that the "Sun Grand" variety is not suitable for growing in sub-Mediterranean Herzegovina, while vaeieties "Caldesi 2000" and "Venus" are recommended for intensive cultivation.

REFERENCES

Baldini E., Scaramuzzi F. (1982): Il pesco, Fruticoltura anni 80, Reda, Roma.

Brady, C. J., (1993): Stone fruit. In: Seymour, G., Taylor, J., Tucker, G. (Eds.), Biochemistry of Fruit Ripening. Chapman and Hall, London, pp. 379–404.

Bulatović S. (1969): Specijalno voćarstvo, Nolit, Beograd.

Fleckinger J. (1945): Notations phénologiques et représentations graphiques du dévelopment des bourgeons de Poirier Congrés de Paris de l'Association francaise pour l'avancement des Sciencies, Paris, p 118.

Forgle, W., Scorza, R. (1982): Sentry Peach. Hort Science, 17, 271–272.

Glišić I., Cerović R., Milošević T., Karaklajić-Stajić Ž. (2008): Fenofaze cvetanja i klijavost polena nekih sorti breskve, Voćarstvo, 42, 163-164, 97-102

Lill, R. E., O'Donaghue, E. M., King, G. A., (1989): Postharvest physiology of peaches and nectarines. Hort. Rev. 11, 413–452

IBGR Rome (1984): Peach descriptors, International board for plant genetic resources.

Medin A. (1998): Breskva, suvremena proizvodnja, Alfa, Zagreb.

Milošević T. (1997): Specijalno voćarstvo, Zajednica za voće i povrće, Beograd.

Mratinić E., Milatović D., Đurović D., Milivojević J. (2003): Pomološke osobine poznih sorti nektarine XVIII Savetovanje unapređenje

Mratinić E. (2012)**:** Breskva, PARTENON M.AM. SISTEM, Beograd.

Nicotra., A., Conte, L., Della Strada, G., Fideghelli, C., Insero, O., Liverani, A., Moser, L. (1994): Monografia di cultivar di pesco, nettarine, percoche, Roma.

Niketić M. (1956): Breskva **-** sortno voće. Zadružna knjiga, Beograd.

Naor A., Hupert H., Greenblat Y., Peres M., Kaufman A., Klein I., (2001): The response of nectarine fruit size and midday stem water potential to irrigation level in stage III and crop load. J. Am. Soc. Hortic. Sci. 126, 140–143.

Odalović A. (2002): Pomološke osobine novih sorti breskve (*Prunus persica* (L.) *Batsch)* u rejonu Ćemovskog polja, Doktorska disertacija, Novi Sad.

Odalović A. (2003): Biološko – proizvodne osobine novih sorti breskve u rejonu Ćemovskog polja. Jugoslovensko voćarstvo, vol. 37, 113-121, Čačak.

Ognjanov V., Mišić P., Ogašanović D., Obradović A., Todorović R. (1993): Novi jugoslovenski sortiment breskve. Jugoslovensko voćarstvo 27, 101-102:55-68.

Özkan Y., Özdil S. (2012): Bazı Nektarin Çeşitlerinin Tokat Ekolojik Koşullarında Gelişme.

Pejkić B. (1982): Breskva, Nolit, Beograd.

Prenkić R., Odalović A. (2007): Proučavanje osnovnih karakteristika novih sorti breskve u srednjem dijelu Crne Gore, Savremena poljoprivreda 56/6, N. Sad, 122-129.

Prenkić R., Odalović A. (1997): Biopomološke i tehnološke osobine važnijih sorti nektarine gajenih u uslovima Podgorice, Poljoprivreda i šumarstvo, Vol.43 (1-2) 1-122, Podgorica.

Slovic, D. (1972): Vocarstvo, Univerzitetski udzbenik, Novi Sad.

Vujanić-Varga D., Ognjenov V. (1990): The Population of Vineyard Peaches in the Region of Fruška Gora Mountain, Genetika, Beograd, 22,1, 37-44.

Vujanić-Varga D., Ognjenov V. (1992): Conservation of Vineyard Peach Population in Yugoslavia, Fao/IBPGR Plant Genetic Resources Newsletter, 90, 28-30.

Vujanić-Varga D., Ognjenov V., Lalić D., Horvat A. (1988): Proučavanje populacije vinogradarske breskve U Fruškoj gori, Jugoslovensko voćarstvo, 22, 84/85, 137-142.

Vujanić-Varga D., Krstić M., Ognjanov V., Gašić K. (2000): Rodnost sorti bresaka najkasnije epohe sazrevanja ploda, Letopis naučnih radova, broj 1-2, strana 25-30

Zec G., Čolić S., Janković Z. (2009): Pomološke karakteristike nektarina poznog zrenja, Originalan naučni rad, Voćarstvo, 43, 165-166 (2009) 31-35.

IMPROVED WALNUT CULTIVAR IDENTIFICATION USING REFERENCE SSR PROFILES*

POBOLJŠANA IDENTIFIKACIJA KULTIVARA ORAHA POMOĆU REFERENTNIH SSR PROFILA

Dinko Bećirspahić[1], Mirsad Kurtović[2], Fuad Gaši[2], Jasmin Grahić[2], Azra Skender[1]

Original scientific paper

Summary

In the last few decades fruit trees were identified on the basis of morphological, physiological and agronomic traits. However, traditional cultivar identification based on morphological traits requires extensive observations of mature plants, and takes a lot of time. The rapid development of molecular markers solved this difficult problem successfully. One of the crucial advantages of molecular markers is the fact that they are not influenced by the plants physiology or environment as is the case with morphological markers. The choice of the marker system depends on the type of genomic information required and their ability to detect polymorphisms in a given population. Microsatellite or simple sequence repeat (SSR) markers have been widely used in agricultural crops because of their easiness of analysis. In order to create a list with reference SSR profiles for the most commonly used and the newly introduced walnut cultivars to Bosnia and Herzegovina (B&H), we analyzed ten international walnut cultivars using ten SSR loci, and determined SSR profiles typical for each cultivar. The gained SSR profile list can be used as a reference in future walnut cultivar identification projects.

Keywords: *walnut, cultivars, microsatellite, markers, SSR profiles*

Rezime

U posljednjih nekoliko desetljeća identifikacija voćaka bazirala se na osnovu morfoloških, fizioloških i agronomskih osobina. Međutim, tradicionalna identifikacija kultivara temeljena na morfološkim osobinama zahtijeva opsežna promatranja odraslih biljaka i traje mnogo vremena. Brzi razvoj molekularnih markera uspješno je riješio ovaj problem. Jedna od značajnih prednosti molekularnih markera jest činjenica da oni nisu pod utjecajem fiziologije biljaka ili okoliša, kao što je slučaj s

---

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Biotechnical faculty of University in Bihać; http://btf.unbi.ba/
[2] Faculty of Agriculture and Food Technologies, Sarajevo; http://www.ppf.unsa.ba/
Correspondence:DinkoBećirspahić, Bihać ; E-mail: dinkoxavi@hotmail.com

morfološkim markerima. Odabir markerskog sistema ovisi o vrsti potrebnih genomskih informacija i njihovoj sposobnosti da detektiraju polimorfizme u određenoj populaciji. Mikrosatelitski ili jednostavne ponavljajuće sekvence (SSR) su markeri široko korišteni u poljoprivrednim kulturama zbog njihove jednostavnosti analiziranja. Da bismo stvorili popis s referentnim SSR profilima za najčešće korištene i novo introducirane kultivare oraha u Bosni i Hercegovini (BiH), analizirali smo deset međunarodnih kultivara oraha pomoću deset SSR lokusa i odredili SSR profili tipični za svaki kultivar. Dobiveni popisi SSR profila mogu se koristiti kao referenca u budućim projektima identifikacije kultivara oraha.

Ključne riječi: *orah, kultivari, mikrosateliti, markeri, SSR profili*

INTRODUCTION

The accurate identification of genotypes represents the basic requirement for the management and use of germplasm (clarification of synonyms, homonyms, and misnaming) for practical breeding purposes and protection of intellectual property. Traditional methods of characterization and assessment of walnut variability are based on the analysis of morphological, physiological and biochemical traits. However, the effect of the environment in the expression of quantitative traits, as well as the long juvenile period can cause difficulties and errors in the process of determination and classification of the analysed walnut genotypes. Molecular markers have been successfully used to overcome these limitations, i.e. differentiation, characterization, and identification of walnut genotypes (Ruiz-Garcia *et al.*, 2011).

In contemporary research, various biochemical and molecular markers were used for the genetic characterisation of walnut. These include isoenzymes (Altea *et al.*, 1990; Altea *et al.*, 1991; Germain *et al.*, 1993; Solar *et al.*, 1994; Fornari *et al.*, 1999, Vyas *et al.*, 2003), restriction fragment length polymorphisms - RFLPs (Fjellstrom and Parfitt, 1994), randomly amplified polymorphic DNAs - RAPDs) (Francesca *et al.*, 2010; Erturk and Dalkilic 2011; Fatahi *et al.*, 2010; Abuin *et al.*, 2002), and amplified fragment length polymorphisms – AFLP (Qingguo *et al.*, 2011).

Due to their high variability, codominance and high informativeness, sequences with simple sequence repeats (SSRs) - SSR markers or microsatellites – are ideal for the research of populations and conservation of genes, genetic cartography, analysis of paternity and relations, as well as the analysis of the variations between species and populations (Karimi *et al.*, 2010). Moreover, Ruiz-Garcia *et al.* (2011) stated that, among DNA based markers, microsatellite markers or SSRs allow a higher level of resolution in genetic research due to their high polymorphism, hereditary co-dominance, reproducibility, and easy detection using PCR. Woeste *et al.*, (2002) have developed a list of 30 nuclear microsatellites (SSR) for a broad range of genetic research of *Juglans* species. In the past twelve years, a high number of studies can be found on the use of microsatellites in the identification of walnut cultivars (Dangl *et* *al.*, 2005; Foroni *et al.*, 2005, 2006 and 2007; Ruiz-Garcia *et al.*, 2011; Pop *et al.*, 2013).

Since modern international walnut cultivars are increasingly introduced to the territory of Bosnia and Herzegovina, the need for identification of guaranteed species purity and the quality of planting material has become an imperative. Therefore, the goal of the present study is the genetic identification of ten most used walnut cultivars on the territory of Bosnia and Herzegovina, i.e. identification of the SSR profiles typical for each cultivar. Next to genetic identification, the goal is to contribute to the completion of the genetic profile database of common walnut cultivars (*Juglans regia* L.).

MATERIAL AND METHODS

Plant material and sample preparation. Ten cultivars of walnut have been examined including two from Turkey (Bilecik and Kaman), two from Serbia (Sampion and Novosadski Kasni), two from Czech Republic (Apolo and Jupiter), two from Bulgaria (Drjanovski i Sheinovo), one from France (Saint-Jean) and one from Slovenia (Elit). All cultivars were obtained from the walnut orchard in Bratunac, Bosnia and Herzegovina.

For DNA extraction, approximately four young leaves were taken from each tree of analyzed cultivars. Leaf gathering was carried out in May 2016. The youngest, healthy leaves from the periphery of the tree top represented individual samples, with the goal of acquiring a high amount of DNA and DNA purity. The leaves were put into marked paper envelopes, which were preserved in the Gen bank of the Faculty of Agriculture and Food Sciences in Sarajevo, in a freezer at -80 °C until extraction.

DNA extraction. A modified CTAB protocol was used for DNA isolation (Doyle and Doyle, 1987; Cullings, 1992). CTAB buffer in the amount of 500 µl was added into each sample of 10-20 mg of pulverized leaf. In order to collect 15 ml of CTAB buffer, the following components were necessary: 1.5 ml 1M Tris, pH 8.0, 4.2 ml 5M NaCl, 600 µl 0.5M EDTA, 0.3 g CTAB, 8.7 ml dd H_2O, 0.6 g PVP, and 75 µl of β-mercaptoethanol. After DNA isolation, the efficacy of DNA isolation was verified by the method of horizontal electrophoresis in 1,5% agarose gel in 1x SB buffer, pH 8 (Brody and Kern, 2004).

SSR primers and PCR amplification. Ten microsatellite primers (Table 1) were used for DNA amplification, which were originally developed for *Juglans nigra* by Woeste *et al.* (2002), and which have found their application later in the studies of genetic diversity and identification of *Juglans regia* units by Dangle *et al.*, (2005); Ruiz-Garcia (2011), and Vahdati *et al.*, (2014).

Amplification of microsatellite sequences was performed in PCR machine ABI GeneAmp® PCR System 9700. Fluorescent-labelled primers were used for amplification due to possibilities of multiplexing and analyzing the PCR product in a

DNA sequencer. Amplification of selected loci was performed in two separate PCR reactions, namely, two multiplexes with five primer pairs each. Both PCR reactions were carried out in a 15 µl volume, containing 25 ng/micl DNA, 50 mM $MgCl_2$, PCR buffer, 10 µM dNTPs, 5 u/µl Taq polymerase (DNA, Gdansk Poland) and 10 µM forward and reverse starters of five microsatellite loci.
The PCR cycling conditions for two separate PCR reactions (mix 1 and mix 2) were the same and represents a modification of the protocol described in Dangle *et al.*, (2005). The first step in the mentioned protocol is the activation of enzymes for 3 min at 94 °C, denaturation (30 sec at 94 °C), overlapping the starters (1 min at 58 °C, 30 cycles), elongation (40 sec at 72 °C) and final elongation for 5 min at 72 °C.
The size of alleles was determined by PCR product analysis in ABI 3500 genetic analyser, by method of vertical capillary electrophoresis. The obtained data was processed with the use of GeneMapper ID 5 software.

Table1. Details and characteristics of ten microsatellite primers used for the analysis of ten international walnut cultivars.

SSR locus	Primer sequence (5´ → 3´)	Range (bp)
WGA1	F: ATTGGAAGGGAAGGGAAATG R: CGCGCACATACGTAAATCAC	180-192
WGA4	F: TGTTGCATTGACCCACTTGT R: TAAGCCAACATGGTATGCCA	226-238
WGA9	F: CATCAAAGCAAGCAATGGG R: CCATTGGTCTGTGATTGGG	231-245
WGA69	F: TTAGATTGCAAACCCACCCG R: AGATGCACAGACCAACCCTC	160-182
WGA89	F: ACCCATCTTTCACGTGTGTG R: TGCCTAATTAGCAATTTCCA	212-222
WGA118	F: TGTGCTCTGATCTGCCTCC R: GGGTGGGTGAAAAGTAGCAA	186-220
WGA202	F: CCCATCTACCGTTGCACTTT R: GCTGGTGGTTCTATCATGGG	259-295
WGA276	F: CTCACTTTCTCGGCTCTTCC R: GGTCTTATGTGGGCAGTCGT	168-194
WGA349	F: GTGGCGAAAGTTTATTTTTTGC R: ACAAATGCACAGCAGCAAAC	262-274
WGA376	F: GCCCTCAAAGTGATGAACGT R: TCATCCATATTTACCCCTTTCG	243-253

Jaccard distance between the analyzed walnut cultivars was calculated and used to construct a phylogenetic tree utilizing the unweighted pair group method with arithmetic mean (UPGMA). Statistical analysis were carried out using the R software version 3.2.3 (R core team, 2016).

RESULTS AND DISCUSSION

All ten used primer pairs have amplified PCR products which were extremely polymorphic, producing positive, uniform and repeatable results in all ten international cultivars. Moreover, the mentioned primer pairs have shown a high level of polymorphism in other similar studies as well (Dangl *et al.*, 2005; Foroni *et al.*, 2005; Ruiz-Garcia *et al.*, 2011; Pop *et al.*, 2013). Reading the products of PCR amplification was relatively easy, representing the most significant trait of these markers next to high polymorphism. PCR products generated with SSR genotyping have produced both homozygous and heterozygous traits, which is in accordance with the diploid level of common walnut *Juglans regia* L. (Table 2). Most of the cultivars studied in this work have not been subjected previously to molecular characterization.

Table 2. SSR profiles of ten international walnut cultivars by using ten SSR markers

Cultivar	**WGA69**		**WGA4**		**WGA1**		**WGA89**		**WGA9**		**WGA118**		**WGA202**		**WGA276**		**WGA376**		**WGA349**	
Apolo	160	178	216	230	190	190	212	220	240	248	198	198	261	273	180	190	252	252	270	270
Drjanovski	160	178	216	228	190	192	216	220	244	244	196	198	257	257	178	190	244	252	270	270
Kaman	160	176	216	228	188	188	216	220	240	240	198	198	261	261	180	180	230	230	270	270
Elit	160	160	216	228	190	192	216	228	248	248	196	198	263	263	178	190	236	252	262	274
Šampion	162	178	228	228	190	192	216	216	240	248	184	198	247	261	176	176	252	252	262	274
Novosadski K.	160	160	228	228	180	192	212	216	232	248	198	206	263	269	172	190	238	244	262	274
Sheinovo	160	176	228	228	190	190	212	216	240	244	196	206	263	273	168	190	238	238	262	274
Saint-Jean	160	162	230	230	192	192	216	220	240	240	196	196	273	273	168	182	238	252	274	274
Bilecik	160	160	228	228	192	196	216	220	244	244	196	206	269	273	172	180	238	244	270	274
Jupiter	178	178	230	230	190	190	212	220	244	248	184	196	263	269	174	174	236	246	274	274

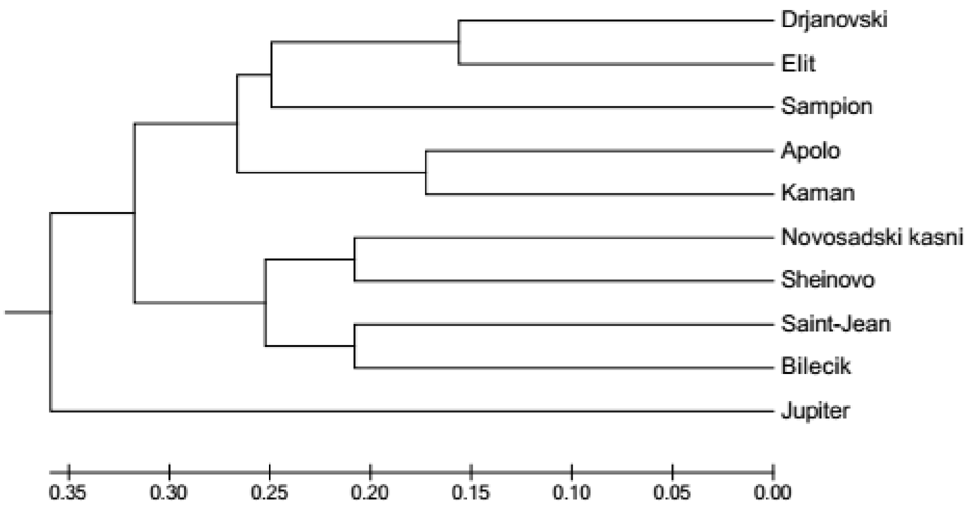


Figure 1. UPGMA cluster analysis based on polymorphism of 10 SSR loci in ten international walnut cultivars using Jaccard distance.

Based on the UPGMA cluster analysis, ten cultivars of walnuts formed three major groups showing genetic similarity on the basis of 10 SSR markers (Figure 1). The first cluster included five (Drjanovski, Elit, Sampion, Apolo and Kaman) and the second one four walnut cultivars (Novosadski kasni, Sheinovo, Saint-Jean and Bilecik). The third cluster was reserved for the Jupiter cultivar that showed less genetic similarity than other cultivars. Although the walnut cultivars analysed in this study originate from six different geographical regions, and larger similarities between cultivars from the same region were expected, no correlation between cluster grouping and place of origin was detected. The fact that some walnut cultivars are more similiar to the cultivars from the other regions than to cultivars of the same origin/same breeding program, indicates that different walnut breeding programs, like those in Bulgaria (Drjanovski and Sheinovo), Slovenia (Elit), Serbia (Sampion and Novosadski kasni), Czech Republic (Apolo and Jupiter), Turkey (Kaman and Bilecik) and France (Saint-Jean), were not only focused on the local genetic material but on the advances of other breeding programs as well. Relationships between the introduced walnut cultivars to B&H must be further investigated.

CONCLUSION

All ten used primer pairs have amplified PCR products which were extremely polymorphic, producing positive, uniform and repeatable results in all ten international cultivars, which points to a good selection of molecular markers.

A typical SSR profile was assigned to each introduced walnut cultivar on the territory of Bosnia and Herzegovina.
The results of this study will be used to supplement the dataset of reference genetic profiles of fruit crops with SSR profiles of common walnut cultivars (*Juglans regia* L.) that are present in Bosnia and Herzegovina.

ACKNOWLEDGEMENT

This study was partially funded by the Federal Ministry of Education and Science, project "Pre-selection of indigenous genotypes of walnuts (*Juglans regia* L.) in Bosnia and Herzegovina" (number 02-8178/15. of 03.12.2015.).

REFERENCE

Abuin, M., Diaz, R., Fernandez-Lopez, J. (2002): Identidication of *Juglans regia* L. genotypes using RAPDs molecular markers. Arbora Publishers. Forest genetics 9(1): 31-37.

Aleta, N., Olarte, C., Truco, M. J., Arus, P (1990): Identification of walnut cultivars by isozyme analysis. Acta Horticulturae 284: 91–96.

Aleta, N., Rovira, M., Ninot, A., Arus, P. (1991): Inheritance of four isozymes in walnut. Acta Horticulturae 311:62–65.

Brody, J. R., Kern, S. E. (2004): History and principles of conductive media for standard DNA electrophoresis. Anal. Biochem; 333: 1–13.

Cullings, K. W. (1992): Design and testing of a plant-specific PCR primer for ecological and evolutionary studies. Molecular Ecology 1: 233-240.

Dangle, G. S., Woeste, K. E., Aradhya, M. K., Koehmstedt, A., Simon, C., Potter, D., Leslie, C. A., McGranahan, G. H. (2005): Characterization of fourteen microsatellite markers for genetic analysis and cultivar identification of walnut. J. Amer. Soc. Hort. Sci. 130:348–354.

Doyle, J., Doyle, J. L. (1987): Genomic plant DNA preparation from fresh tissue-CTAB method. Phytochem. Bull, 19: 11–15.

Erturk, U., Dalkilic, Z. (2011): Determination of genetic relationship among some walnut (*Juglans regia* L.) genotypes and their early-bearing progenies using RAPD markers. Rom. Biotech. Lett. 16: 5944–5952.

Fatahi, R., Ebrahimi, A., Zamani, Z. (2010): Characterization of some Iranians and foreignwalnut genotypes using morphological traits and RAPD markers. Hortic. Environ. Biotechnol. 51: 51–60.

Fjellstrom, R. G., Parfitt, D. E., McGranahan, G. H. (1994): Genetic relationship and characterization of Persian walnut (*Juglans regia* L.) cultivars using restriction fragment length polymorphisms (RFLPs). J Amer Soc Hort Sci 119: 833-839.

Fornari, B., Malvolti, M. E., Taurchini, D., Fineschi, S., Beritognolo, I., Maccaglia, E., Cannata, F. (1999): Isozyme and organellar DNA analysis of genetic diversity in natural/naturalised European and Asiatic walnut (*Juglans regia* L.) populations. Acta Hort. 544:167–178.

Foroni, I., Rao, R., Woeste, K., Gallitelli, M. (2005): Characterization of *Juglans regia* L. with SSR markers and evaluation of genetic relationships among cultivars and the 'Sorrento' landrace. J Hort Sci Biotech 80: 49-53.

Foroni, I., Rao, R., Woeste, K., Gallitelli, M. (2006): Molecular Characterization of *Juglans regia* L. Cultivars with SSR Markers. Acta Hort (ISHS) *705*: 207-213.

Foroni, I., Woeste, K., Monti, M. L., Rao, R. (2007): Identification of 'Sorrento'walnut using simple sequence repeats (SSRs). Genet Resour Crop Evol 54: 1081-1094.

Francesca, PI., Pamfil, D., Raica, P. (2010): Assessment of the genetic variability among some *Juglans* cultivars from the Romanian National Collection at SCDP Valcea using RAPD markers. Rom Biotech Lett 15: 41-49.

Germain, E., Hanquier, I., Monet, R. (1993): Identification of eight *Juglans* sp. and some inter-specific hybrids by isoenzyme patterns. Acta Horticulturae 311:73–81

Karimi, R., Ershadi, A., Vahdati, K., Woeste, K. (2010): Molecular characterization of Persian walnut populations in Iran with microsatellite markers. Hortscience 45: 1403–1406.

Pop, I., Vicol A., Botu, M., Raica, P., Vahdati, K., Pamfil, D. (2013): Relationships of walnut cultivars in a germplasm collection: Comparative analysis of phenotypic and molecular data. Scientia Horticulturae 153: 124–135

Qingguo, M., Zhang, J., Dong., P. (2011): Genetic Analysis of Walnut Cultivars in China Using Fluorescent Amplified Fragment Length Polymorphism. J. Aamer. Soc. Hort. Sci. 136(6):422–428.

R Core Team (2016): R - A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Ruiz-Garcia, L., Lopez-Ortega, G., Denia, A. F., Tomas, D. F. (2011): Identification of a walnut (*Juglans regia* L.) germplasm collection and evaluation of their genetic variability by microsatellite markers. Span. J. Agric. Res. 9: 179–192.

Solar, A., Smole, J., Stampar, F. Viricek-Marn, M. (1994): Characterization of isozyme variation in walnut (*Juglans regia* L.). *Euphytica* 77: 105-112.

Vahdati K., Pourtaklu S. M., Karimi R., Barzehkar R., Amiri R., Mozaffari M., Woeste K. (2014): Genetic diversity and gene flow of some Persian walnut populations in southeast of Iran revealed by SSR markers. Plant Systematics and Evolution 301(2): 691–699.

Vyas, D., Sharma, S. K., Sharma, D. R. (2003): Genetic structure of walnut genotype using leaf isozymes as variability measure. Sci. Hort. 97:141–152.

Woeste, K., Burns, R., Rhodes, O., Michler, C. (2002): Thirty polymorphic nuclear microsatellite loci from black walnut. J Hered 93: 58-60.

UTJECAJ KRUPNOĆE SJEMENA I HEMIJSKOG TRETMANA HIDROGEN PEROKSIDOM (H_2O_2) NA KLIJAVOST SJEMENA I OSOBINE SIJANACA PITOMOG KESTENA*

THE EFFECT OF SEED SIZE AND CHEMICAL TREATMENT OF HYDROGEN PEROXIDE (H_2O_2) ON THE SEED GERMINATION AND CHARACTERISTICS OF SEEDLING OF SWEET CHESTNUT

Fikreta Behmen[1], Haris Mašinović[1], Senad Murtić[1], Mersija Delić[1], Pakeza Drkenda[1], Osman Musić[1]

Originalan naučni rad

Rezime

U ovom istraživanju proučavani su uticaji krupnoće sjemena i hemijskog tretmana hidrogen peroksidom na klijavost sjemena i rast sijanaca *Castanea sative* Mill. Sjeme je klasificirano u tri grupe male < 1g, srednje > 2g i velike > 4g. Prilikom ispitivanja klijavosti sjemena u zavisnosti od krupnoće sjemena, najveću je imalo sjeme iz grupe <1g (80%). Kod sjemena tretiranog različitim koncentracijama H_2O_2, najveću klijavost je imalo sjeme tretirano sa 1M H_2O_2 (76,7%). Kod ispitivanja krupnoće sjemena na morfološka svojstva sijanca, sjeme iz grupe > 4g za sve ispitivane parametre imalo je veće vrijednosti u odnosu na sjemena iz grupa < 1g i > 2g. Učinkovitost hidrogen peroksida imala je samo pozitivan efekat u mjerenju mase korijena.

Ključne riječi: *kesten, krupnoća sjemena, hidrogen peroksid, klijavost, korijen, morfološka svojstva*

Summary

The effects of seed size and chemical treatment of hydrogen peroxide on seed germination, and seedling survival and growth of *Castanea sativa* Mill. were studied in this study. The seeds were classified into small < 2g, medium > 2g and large > 4g classes. When seed germination was tested depending on seed size, the largest seed of group <1g (80%) was obtained. For seeds treated with different concentrations of H_2O_2, the highest germination had the seed treated with 1M H_2O_2 (76.7%). The effect of seedling size on the morphological traits of chestnut seedlings is evident and the results show that the larger fruits (>4g) have statistically significant differences in the obtained morphological traits compared to the smaller seeds (1g and 2g). Effectiveness of hydrogen peroxide was observed only in measuring the mass of roots.

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina

[1] Poljoprivredno-prehrambeni fakultet Univerziteta u Sarajevu/Faculty of Agriculture and Food Sciences Univerity of Sarajevo

Key words: *chestnut, seed size, hydrogen peroxide, germination ,root, morfological traits*

UVOD

Pitomi kesten, *Castanea sativa* Mill., u Bosni i Hercegovini ima široku ekološku rasprostranjenost. Upravo iz ovako velikog ekološkog areala, u kojem je moguća uspješna proizvodnja, proističe značaj pitomog kestena. Kod nas je još uvijek šumska vrsta i najveća proučavanja izvršili su stručnjaci iz šumarstva, s ciljem racionalnije i intenzivnije eksploatacije kestena kao brzorastućeg šumskog drveta.

Sadnice pitomog kestena proizvode se generativno (sjetvom plodova u kojima se nalazi jedan ili više sjemenih zametaka) i vegetativno (izdancima i kalemljenjem). Sijanci kestena su pogodni za pošumljavanje, a mogu se koristiti i kao podloga za kalemljenje sorti kestena krupnijih plodova. Rasadnici malo proizvode ovaj sadni materijal iz više razloga, ali prije svega slabija klijavost sjemena, slabiji prijem prilikom kalemljenja a samim tim je i materijalna dobit manja, kao i drugi problemi s kojima se rasadničari susreću prilikom proizvodnje sadnog materijala kestena.

Objavljeno je dosta rezultata o uticaju veličine sjemena na klijavost i rast različitih vrsta, i može se reći uopšteno da su veličina sjemena, klijavost sjemena i rast sadnica u pozitivnoj korelaciji (Singh *et al.*, 1993; Ke i Werger, 1999., Navarro *et al.*, 2006). Drugi autori su ispitujući klijavost sjemena nekih vrsta, utvrdili da krupnije sjeme nije imalo najveći procenat klijavosti (Chaisurisri *et al.*, 1992; Jayasankar *et al.*, 1999; Tilki i Alptekin, 2005); opstanak sijanaca i rast sadnica (Krishan *et al.*, 1995; Indira *et al.* , 2000).

Vrlo malo je dostupno informacija o uticaju veličine sjemena na klijavost sjemena i rast sadnica pitomog kestena. Stoga je i jedan od ciljeva ovog istraživanja bio utvrditi uticaj veličine sjemena na klijavost sjemena i rast sijanaca pitomog kestena.

Primjenom hidrogen peroksida (H_2O_2) odgovarajuće koncentracije može se povećati postotak klijavosti sjemena kao i ubrzati usporeni rast sadnica (Barba - Espin *et al.*, 2010). Predhodno tretiranje hidrogen peroksidom, ekstracelularni ROS (Reactive Oxygen Species) se značajno prevladava odlaganje klijavosti pod uticajem povećanog nivoa soli i temperature, takođe je značajno ublažilo inhibiciju soli na rast sadnica na svim temperaturama (Cavusoglu and Kabar, 2010).

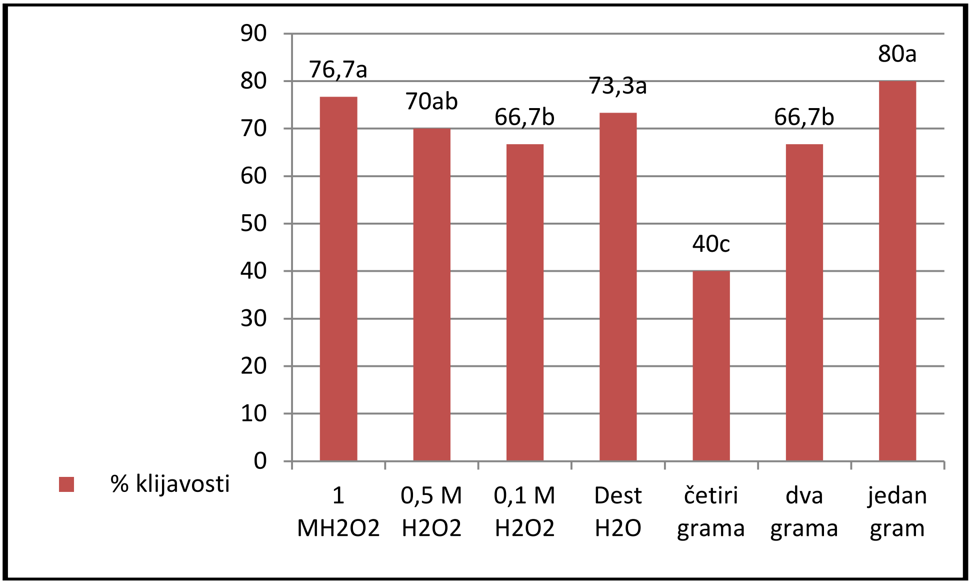
U radu su predstavljeni rezultati uticaja hidrogen peroksida (H_2O_2) i krupnoće sjemena na klijavost pitomog kestena gdje su korištene različite koncentracije H_2O_2 i različite mase sjemena, te analiziran njihov uticaj na morfološke osobine sijanaca: visina nadzemnog sistema, dužina korijena, masa nadzemnog dijela, masa korijena i promjer korjenovog vrata.

MATERIJAL I METOD RADA

Uzorci sjemena koji su korišteni u ovom radu prikupljeni su na području opštine Velika Kladuša, selo Šestenovac koje se nalazi na nadmorskoj visini od 150 m, gdje preovladavaju listopadne šume. U mjesecu novembru 2014 god. prikupljeno je sjeme kestena, zatim je oprano i odstranjeno prazno i deformisano sjeme. Sjeme je stavljeno u plastičnu posudu prekrivenu papirnim maramicama. Papirna maramica se stalno natapala sa vodom. Tako uskladišteno sjeme kestena čuvano je mjesec dana u tamnoj prostoriji na temperaturi koja se kretala do +4 °C. Nakon prikupljanja i pripreme uzoraka izvršeno je grupisanje plodova u 4 grupe po 30 plodova (Roach T., 2009). Svaka grupa je zasebno tretirana sa po 400 ml H_2O_2 u sljedećim koncentracijama: H_2O_2 1 M H_2O_2; 0,5 M H_2O_2; 0,1 M H_2O_2 i jedna grupa (kontrolna) sa 400 ml destilovane vode i nakon toga posijano u plastične žardinjere koje su ispunjene supstratom FLORAHUM-S. Nakon toga je postavljena druga varijanta ogleda sa po 30 plodova u 3 grupe po težini 1 g, 2 g i 4 g. Svaka grupa posijana je u plastične žardinjere koje su ispunjene sa supstratom FLORAHUM-S.
Po završetku vegetacije izvršeni su proračuni klijavosti procentualno, tako što su brojani plodovi koji su isklijali. Nakon toga izvršeno je vađenje sijanaca na kojima su urađena mjerenja morfoloških svojstava: visina nadzemnog sistema, dužina korijena, masa nadzemnog dijela, masa korijena i promjer korijenovog vrata.
Mjerenje dužine je obavljeno metrom, a masa automatskom vagom. Debljina korjenovog vrata se mjerila šublerom sa automatskim mikrometrom.
Rezultati ispitivanja obrađeni su analizom varijanse (ANOVA), a u slučajevima kada se F vrijednost pokazala kao statistički značajna, razlike srednjih vrijednosti između grupa testirane su sa LSD testom. Za statističku obradu podataka dobivenih mjerenja korišten je programski paket (softver) *Excel SPC Software - QI Macros.*

REZULTATI I DISKUSIJA

U skladu sa postavljenim ciljevima da se ispita uticaj tretmana različitih koncentracija H_2O_2 na klijanje sjemena kestena, te uticaj krupnoće sjemena na klijavost, vršeni su proračuni klijavosti, izraženi procentualno. Rezultati računati na osnovu 30 sjemenki u svakoj grupi, su slijedeći:



Graf. 1. Procentualna klijavost sjemena kestena / *Fig. 1. Germination percentage of chestnut seed*

Razlike između uzoraka sa istim slovnim oznakama nisu statistički značajne / *Values followed by same latter in a row are not significantly different*

Prema dobijenim rezultatima iz grafikona 1. može se vidjeti da, posmatrano zasebno sjeme tretirano sa različitim koncentracijama H_2O_2, najveći procenat klijavosti ima sjeme tretirano sa koncentracijom 1M H_2O_2, dok najmanji procenat klijavosti ostvarila je grupa sjemena tretirana sa 0,1 M H_2O_2.

Prema Ashraf i Foolad (2005); akumulacija H_2O_2 i ostalih ROS tokom skladištenja omogućuje klijanje ali ima i štetne učinke na održivost sjemena.

Posmatrajući klijavost sijanaca u odnosu na krupnoću sjemena, najveću klijavost je ostvarilo sjeme od 1 g, što nije u skladu sa nekim istraživanjima prema kojima se tvrdi da plodovi veće krupnoće imaju bolju klijavost. Prema Cicek and Tilki, (2007) klijavost u mnogome zavisi od krupnoće ploda i plodovi s masom većom od 8 g ranije klijaju i imaju bolju klijavost u odnosu na sitnije plodove.

Dakle, rezultati pokazuju da je sjeme od 4g (najveće krupnoće) imalo najmanji procenat klijavosti.

Tabela 1. Uticaj krupnoće sjemena kestena i tretiranja različitim koncentracijama hidrogen peroksida na ispitivane morfološke osobine sijanaca
Table 1. Effects of the treatment of chestnut seed with different concentrations of hydrogen peroxide on the examined morphological traits and chestnut seed size effects on the examined morphological traits

Ogledni faktori		Parametar				
		Visina stabla (mm)	Dužina korijena (mm)	Masa nadzemnog dijela (g)	Masa korijena (g)	Promjer korjenovog vrata (mm)
Hidrogen peroksid	***1 M H_2O_2***	16,18[a]	37,93[a]	2,64[a]	21,95[a]	6,51[a]
	0,5 M H_2O_2	18,98[a]	39,4[a]	4,18[a]	17,52[ab]	7,66[a]
	0,1 M H_2O_2	15,88[a]	35,07[a]	3,48[a]	12,13[abc]	7,25[a]
	Dest H_2O	16,38[a]	34,13[a]	2,31[a]	6,45[bcd]	5,95[a]
F		NS	NS	NS	*	NS
$LSD_{0,01}$					13,28	
Krupnoća sjemena	**4g**	25,23[a]	35,73[a]	4,18[a]	8,8[a]	6,95[a]
	2g	21,45[ab]	29,38[ab]	2,12[bc]	3,85[bc]	4,95[bc]
	1g	17,45[bc]	22,83[bc]	2,22[b]	4,58[b]	5,03[b]
F		*	*	*	*	*

* significant at $p \leq 0{,}01$; *NS- not significant*
Razlike između uzoraka sa istim slovnim oznakama nisu statistički značajne / *Values followed by same latter in a row are not significantly different*

U tabeli 1. su predstavljeni rezultati ispitivanja uticaja različitih koncentracija H_2O_2 na morfološke osobine sijanca pitomog kestena (visinu stabla, dužinu korijena, masu nadzemnog sistema, masu korijena i promjer korjenovog vrata).
Kod ispitivanja uticaja H_2O_2 dobijeni rezultati samo su u jednom slučaju statistički značajni i to prilikom tretiranja koncentracijom 1M H_2O_2. Značajnost se ogleda na parametar masu korijena.
Prema El-Maarouf–Bouteaui Bailly, (2008) predtretmani sjemena sa određenim koncentracijama ROS, a naročito sa H_2O_2 imaju pozitivan uticaj na klijavost sjemena kao i na daljni razvoj biljke (antistres, bolje morfološke osobine i dr.).

Kod ispitivanja uticaja krupnoće sjemena na morfološke osobine sijanaca (visinu stabla, dužinu korijena, masu nadzemnog sistema, masu korijena i promjer korjenovog vrata) dobijeni su sasvim drugačiji rezultati. Naime, za sve ispitivane parametre postoji statistički značajna razlika. Sjeme kestena krupnoće 4 gr je u svim slučajevima pokazalo bolje rezultate u odnosu na sjeme 1 i 2 g.
Prema Cicek and Tilki, (2007) klijavost i energija klijanja u mnogome zavisi od krupnoće plodova. Plodovi sa masom preko 8 grama ranije klijaju i imaju bolju klijavost u odnosu na sitnije plodove. Krupnoća plodova značajno utječe na izbijanje klice i opstanak klijanca, a manji je utjecaj na morfološke osobine sijanaca.

.

ZAKLJUČAK

Prema postavljenim ciljevima i zadacima rada da se ispita uticaj tretmana različitih koncentracija H_2O_2 i krupnoće sjemena na klijavost sjemena kestena i neka morfološka obilježja sijanaca, donešeni su sljedeći zaključci:

- Prilikom ispitivanja procentualne klijavosti sjemena u zavisnosti od krupnoće, najveću klijavost imalo je sjeme <1g (80%),
- Sjeme predhodno tretirano sa najvećom koncentracijom 1M H_2O_2 imalo je najveću procentualnu klijavost (76,7%)
- Kod praćenja morfoloških osobina sjeme najveće krupnoće <4g, imalo je značajan statistički uticaj na ispitivane parametre u odnosu na druge dvije grupe sitnijeg sjemena, što je bilo i za očekivati,
- Primjena različitih koncentracija hidrogen peroksida na sjeme kestena nije imala značajan uticaj na mjerene morfološke osobine, osim na **masu korijena**, gdje je primjena 1 M H_2O_2 pokazala statistički značajne rezultate.

S obzirom na dobijene rezultate može se dati jedan generalni zaključak:
Za proizvodnju generativno razmnoženih sadnica kestena treba koristiti što krupnije sjeme. Kada je u pitanju hidrogen peroksid ogled treba nastaviti ali sa većim koncentracijama od 1 M H_2O_2.

LITERATURA

Alptekin, C. and F. Tilki (2002). Effects of stratification and pericarp removal ongermination of *Quercus libani* acorns. Silva Balc., 2: 21-28.

Ashraf, M. Foolad, M. R. (2005). Pre-sowing seed treatment--a shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. Advances in Agronomy 88:223-271

Barba-Espin G., Diaz-Vivancos P., Clemente-Moreno MJ., Albacete A., Faize L. Faize M., Pérez-Alfocea F., Hernández JA. (2010). Interaction between hydrogen peroxide and plant hormones during germination and the early growth of pea seedlings. Plant Cell Environ.; 33(6):981-94.

Çavuşoğlu, K. and Kabar, K. (2010). Effects of hydrogen peroxide on the germinationand early seedling growth of barley under NaCl and high temperature stresses. *EurAsian Journal of Biosciences*, pp. 70-79.

Cicek Emrah, Fahrettin TİLKİ (2007). Seed Size Effects on Germination, Survival and Seedling Growth of Castanea sativa Mill. Journal of Biological Sciences 7(2)

Chaisurisri, K., D. G. W. Edwards and Y. A. El-Kassaby, 1992. Genetic control of seed size and germination in Sitka spruce. Silvae Genet., 41: 348-355.

El-Maarouf-Bouteau H., Bailly C. (2008). Oxidative signaling in seed germination and dormancy. Plant Signal Behav.; 3(3):175-82.

Indira, E. P., S. C. Basha and K. C. Chacko (2000). Effect of seed size grading on the germination and growth of teak (*Tectona grandis*) seedlings. J. Trop. For. Sci., 12: 21-27.

Jayasankar, S., L. C. Babu, K. Sudhakara and V. K. G. Unnithan, (1999). Provenance variation in seed and germination characteristics of teak (*T. grandis* L. F.). Seed Sci. Technol., 27: 131-139.

Ke, G. and M. J. A. Werger, (1999). Different responses to shade of evergreen and deciduous oak seedlings and the effect of acorn size. Acta Oecol., 20: 579-586.

Krishan, B., V. Singh and B. Krishan, (1995). Effects of seed size and colour on germination and seedling growth in five tree species. Adv. Hortic. For., 4: 199-204.

Navarro, F. B., M. M. Jimenez, M. A. Ripoll, E. Ondono, E. Gallego and E. Simon, (2006). Direct sowing of holm oak acorns: Effects of acorn size and soil treatment. Ann. For. Sci., 63: 961-967.

Shepard, E., D. D. Miller, G. Miller and D. Miller, (1989). Effect of weight on emergence and seedling vigor of Chinese chestnut. Hortic. Sci., 24: 516-519.

Singh, S., O. Singh and V. Singh, (1993). Effect of seed weight on germination, survival and initial growth of horse chestnut (*Aesculus indica* Colebr.) in the nursery. Indian For., 119: 627-629.

Tilki, F. and C. U. Alptekin, (2005). Variation in acorn characteristics in three provenances of *Quercus aucheri* Jaub. et Spach and provenance, temperature and storage effects on acorn germination. Seed Sci. Technol., 33: 441-447.

Thomas Roach (2009). Ekstracellular production of reactive oxigen species in response to abiotic stress in seeds. Doctoral thesis.

MORPHOLOGICAL AND CHEMICAL TECHNOLOGICAL PROPERTIES OF SELF - SOWN GENOTYPES OF MULBERRY IN NORTH WESTERN BOSNIA*

MORFOLOŠKA I HEMIJSKO – TEHNOLOŠKA SVOJSTVA SAMONIKLIH GENOTIPOVA DUDA NA PODRUČJU SJEVEROZAPADNE BOSNE

Azra Skender[1], Vildana Alibabić[1], Mirsad Kurtović[2], Edina Šertović[1], Melisa Oraščanin[1], Dinko Bećirspahić[1]

Original scientific paper

Summary

In Bosnia and Herzegovina, mulberry is a self - sown fruit species, and for now there is no data on its intensive breeding. For this reason, mulberry fruit is minimally represented in nutrition, and research on this fruit species is very scarce. The aim of this study was to examine the basic morphological and chemical–technological properties of self–sown genotypes of black and white mulberry in the North-Western Bosnia. The study found that the genotypes stand out for the fruit size and the chemical composition. Using the ANOVA statistical method, it has been found that there is a statistically significant difference ($p < 0.05$) between the genotypes in terms of morphological and chemical– technological properties. The results of the chemical analysis showed that water content in mulberry ranged from 82.8 to 87% and dry matter content ranged from 13.09 to 17.11%. The content of acids ranged from 0.074 to 0.560%, and the sugar content ranged from 4.40 to 7.97%. It is necessary to continue and extend the research of natural populations of black and white mulberry in our area in order to single out valuable genotypes as well as to select and breed this species.

Key words: *mulberry, genotypes, North-Western Bosnia, morphometrics, chemical-technological properties*

Rezime

U Bosni i Hercegovini dud je samonikla voćna vrsta i za sada ne postoje podaci o njegovom intenzivnom uzgoju. Zbog toga je plod duda minimalno zastupljen u ishrani, a i istraživanja o ovoj voćnoj vrsti su vrlo oskudna. Cilj ovog istraživanja bio je ispitati osnovna morfološka i hemijsko–tehnološka svojstva samoniklih genotipova

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] University of Bihać Biotechnical Faculty
[2] University of Sarajevo Faculty of Agriculture and Food Sciences

crnog i bijelog duda na području sjeverozapadne Bosne. Ispitivanjem je utvrđeno da se po veličini ploda i hemijskom sastavu znatno ističu neki od selekcionisanih genotipova. Primjenom ANOVA statističke metode utvrđeno je da između genotipova samoniklog duda postoji statistički značajna razlika ($p<0,05$) u pogledu morfoloških i hemijsko–tehnoloških svojstava. Rezultati hemijske analize su pokazali da se sadržaj vode kretao u rasponu od 82,8 do 87 %, a sadržaj suhe tvari u rasponu 13,09 do 17,11 %. Sadržaj kiselina je bio u rasponu od 0,074 do 0,560 %, a sadržaj šećera u rasponu od 4,40 do 7,97 %. Potrebno je nastaviti i proširiti istraživanja prirodnih populacija crnog i bijelog duda kod nas, kako bi se izdvojili vrijedni genotipovi i vršila selekcija i oplemenjivanje ove vrste.

Ključne riječi: *dud, genotipovi, sjeverozapadna Bosna, morfometrija, hemijsko-tehnološka svojstva*

INTRODUCTION

The coloured fruit is a good source of phenolic compounds, including anthocyanins, flavonoids and carotenoids (Ozgen *et al.*, 2009; Ercisli and Orhan, 2007a; Gerasopoulos and Stavroulakis, 1997; Mikulic – Petkovsek *et al.*, 2012; Skender *et al.*, 2015; Kurtović *et al.*, 2016). Mulberry fruits are rich in phenols and have a unique sour and refreshing taste (Imran *et al.*, 2010; Kutlu *et al.*, 2011; Gecer *et al.,* 2016). They are used as a folk remedy for the treatment of tooth diseases, diabetes, hypertension, arthritis and anaemia (Sass – Kiss *et al.,* 2005). In order to find new sources of natural antioxidants, research of various types of fruits and vegetables are conducted so far, as well as other plants known to possess antioxidant activity (Stinzing *et al.*, 2002; Zadernowski *et al.*, 2005; Ordom *et al.*, 2006). Fresh mulberry fruits and their extracts are rich in phenolic compounds, have high antioxidant activity, show antibacterial activity and can be used in nutrition and preparation of auxiliary pharmaceutical formulations (Radojković *et al*., 2012; Dimitrijević, 2014; Sanches *et al.*, 2014). Mulberry (*Morus sp.*) is a significant species, a deciduous tree from *Moraceae* family. It originates from Asia, and numerous varieties have been produced over the centuries, so today there are many varieties and subtypes of genus *Morus* (Datta, 2000) available on the market. Considering all the economic potentials, much effort has been invested in the cultivation of mulberry with better yields and better adaptability (Ercisli and Orhan, 2007b). More recently, the importance of *M. nigra* variety has increased, as it is believed to be one of the healthiest mulberry fruits due to its high bioactive content (Ercisli and Orhan, 2008; Eyduran *et al*., 2015). In Bosnia and Herzegovina, mulberry is a self-sown fruit species, and for now there are no data on its intensive breeding. For this reason, mulberry fruit is minimally represented in the diet, and research on this fruit type in our country is very scarce. Although it is at the very top of the nutritionally valuable food scale, it is minimally represented in nutrition. The North - Western area of Bosnia and Herzegovina has a very rich genofund of autochthonous fruit genotypes, so thus it has significant genetic resources of mulberry (Skender *et al*., 2014). The objective of this study was to evaluate and compare, for the first time, white and black mulberry species in terms of their main morphological and chemical characteristics in eight genotypes from the North-Western Bosnia.

MATERIALS AND METHODS

During the vegetation period in 2015, samples of autochthonous genotypes of black and white mussels were collected in the area of North-Western Bosnia. The genotypes which were in full fruitfulness were selected. Four black and four white mulberries were selected; 1 kg of fresh fruit was taken from each tree. The fruits were then delivered to the laboratory where the analysis was performed. Thirty fruits were taken for the morphometric analysis of fruit. Analysis of the morphometric characteristics of the fruit was carried out: fruit weight, fruit height, fruit width, and stem length. Also, we determined basic chemical–technological properties of fruit: water content, total soluble solid (TSS) of dry matter, sugar and acidity. Fruit weight was measured by using a digital balance with a sensitivity of 0.001 g (Milivojević *et al*., 2009). Measurement of fruit length, fruit width and stem length was performed by a sliding scale (Milivojević *et al*., 2009). The analysis of chemical–technological properties of mulberry fruit was conducted using AOAC methodology. The experiment had a completely randomized design with four replications. Data were subjected to analysis of variance (ANOVA) and means were separated by Duncan′s multiple range test at $p \geq 0{,}05$ significance level. To determine the similarities or differences of the analysed genotypes, the PCA (*Principal Component Analysis*) was used. The obtained data were processed using the computer statistical program Past (Hammer *et al*., 2001) and XLSTAT 13.

RESULTS AND DISCUSSION

This paper investigates morphological characteristics of two mulberry varieties (*Morus alba* and *Morus nigra*), and four genotypes of each variety were considered. The analysed pomological characteristics of fruit of the examined self-sown mulberry genotypes are shown in Table 1. White mulberry genotypes are labelled as MA1, MA2, MA3 and MA4. Black mulberry genotypes are labelled MN1, MN2, MN3 and MN4.

Table 1. Morphological characteristics of investigated mulberry genotypes

	Fruit length (mm)	Fruit width (mm)	Stem length (mm)	Fruit weight (g)
MA1	15.8 ± 1.95^{b}	10.24 ± 0.89^{e}	12.27 ± 1.67^{a}	0.527 ± 0.14^{f}
MA2	16.9 ± 3.11^{a}	11.17 ± 1.01^{d}	7.6 ± 2.06^{b}	0.83 ± 0.27^{d}
MA3	12.67 ± 1.24^{c}	9 ± 0.7428^{f}	7.24 ± 1.42^{b}	0.425 ± 0.11^{f}
MA4	15.06 ± 1.17^{b}	10.16 ± 0.83^{e}	10.16 ± 1.69^{b}	0.64 ± 0.12^{ef}
MN1	12.37 ± 1.17^{cd}	20.36 ± 1.74^{a}	10.17 ± 1.69^{b}	1.35 ± 0.39^{b}
MN2	12.53 ± 1.061^{c}	21.06 ± 1.74^{a}	10.66 ± 1.72^{b}	1.49 ± 0.22^{a}

MN3	11.66 ±1.008[d]	18.86 ± 1.92[b]	8.86 ± 1.83[c]	1.15 ± 0.36[c]
MN4	10.10 ±0.958[e]	15.63 ± 1.81[c]	8.73± 2.02[c]	0.66 ± 0.24[e]

* Different lower–case letters indicate a significant difference between varieties at p < 0,05 according to Duncan's test

The results showed that there was a statistically significant difference between the analysed genotypes of black and white mulberry in all analysed morphological parameters of fruit. The genotype MN2 (1.49 g) had the highest fruit weight, and MA3 the smallest (0.425 g). The obtained results of fruit weight can be compared to the results of previous studies. It is apparent from literature that the weight of black mulberry fruit ranges from 0,62 g to 6.78 g (Koyuncu *et al.*, 2004; Ercisli and Orhan, 2008; Holecyova *et al.*, 2009; Singhal *et al.*, 2010; Yilmaz *et al.*, 2012; Predojević *et al.*, 2012; Gecer *et al.*, 2016; Aljane and Sdiri 2016). Previous research indicates that the weight of white mulberry fruit ranges from 0.66 g to 3.49 g (Yilmaz *et al.*, 2012; Predojević *et al.*, 2012; Gecer *et al.*, 2016; Aljane and Sdiri, 2016). The width of white mulberry fruit is somewhat smaller (9.00 – 11.17 mm) and the length somewhat bigger (12.67 – 16.9 mm) in comparison to black mulberry (15.63 – 21.06 mm; 10.10 – 12.53 mm). This yields to a conclusion that the fruit of white mulberry is more of an elongated shape than that of black mulberry. Thus, these two botanical species differ in fruit shape. Stem length of fruit varied from 8.73 to 10.66 mm in black mulberry, and 7.24 - 12.27 mm in white mulberry.

Table 2. Chemical characteristics of investigated mulberry genotypes

	Moisture (%)	Dry matter (%)	Acid (%)	Total Sugars (%)
MA1	86.97 ± 0.32[a]	13.09 ± 0.399[d]	0.466 ± 0.291[abc]	7.706 ± 0.5372[a]
MA2	86.87 ± 0.415[a]	13.12 ± 0.480[d]	0. 60 ± 0.355[a]	4.400 ± 0.200[c]
MA3	87.006 ± 0.56[a]	13.11 ± 0.225[d]	0.436 ± 0.240[abc]	5.666 ± 0.503[b]
MA4	86.25 ± 0.317[a]	13.22 ± 0.341[d]	0.480 ± 0.262[ab]	6.266 ± 0.288[b]
MN1	84.226 ± 1.13[bc]	15.77 ± 1.132[ab]	0.158 ± 0.008[bc]	7.600 ± 7.350[a]
MN2	85.85 ± 0.555[ab]	14.14 ± 0.55[cd]	0.16 ± 0.004[bc]	7.97 ± 7.825[a]
MN3	84.53 ± 0.678[b]	15.46 ± 0.68[bc]	0.074 ± 0.016[c]	7.686 ± 7.51[a]
MN4	82.87 ± 1.94[c]	17.11 ± 1.940[a]	0.107 ± 0.008[bc]	7.893 ± 7.72[a]

* Different lower – case letters indicate a significant difference between varieties at p < 0,05 according to Duncan's test

The results of the analysis of the chemical characteristics of the investigated genotypes of black and white self-sown mulberry reveal statistically significant differences (Table 2). The value of moisture content in samples of black and white mulberry ranged from 82.87% (MN4) to 86.97% (MA1). The content of soluble solids, total acids, and sugars differed significantly between genotypes of black and white mulberry. The values of dry matter content ranged from 13.09 to 13.22% (*M. alba*) and 14.14 to 17.11% (*M. nigra*). The obtained values are in line with previous research. In fruits of black mulberry, Koyuncu *et al.* (2004) found 13.11 - 16.23%, Ercisli and Orhan (2007a) found 16.7%, Ercisli and Orhan (2008) found 14.3 - 19.35%, Ercisli *et al.* (2010) found 16.95% - 18.40%, Yilmaz *et al.* (2012) found 17.33 - 21.17% and Okatan *et al.* found 15.65 - 22.10%. White mulberry genotypes did not differ in water content and dry matter content. The content of dry matter (13,09 - 13,22%) was somewhat lower than the value in previous studies, which was 20.40% (Ercisli and Orhan, 2007a); the highest content of total sugars was found in black mulberry genotypes MN4 (7.89%) and MN2 (7.97%), and the lowest in genotype of white mulberry MA2 (4.40%). Sugar content in mulberry fruit is a very important chemical-technological property, because usefulness of mulberry depends on it. Thus, this feature was previously investigated by many scientists: Koyuncu *et al.* (2004), Predojević *et al.* (2012), Mahmood *et al.* (2012), Eyduran *et al.* (2015) and Gecer *et al.* (2016). They found from 5.90 to 17.65% of total sugars in fruits of black mulberry, and 5.90 - 16.00% in white mulberry. The total acids content was higher in white mulberry fruits, where MA2 (0.56%) and MA4 (0.480%) genotypes particularly stand out.

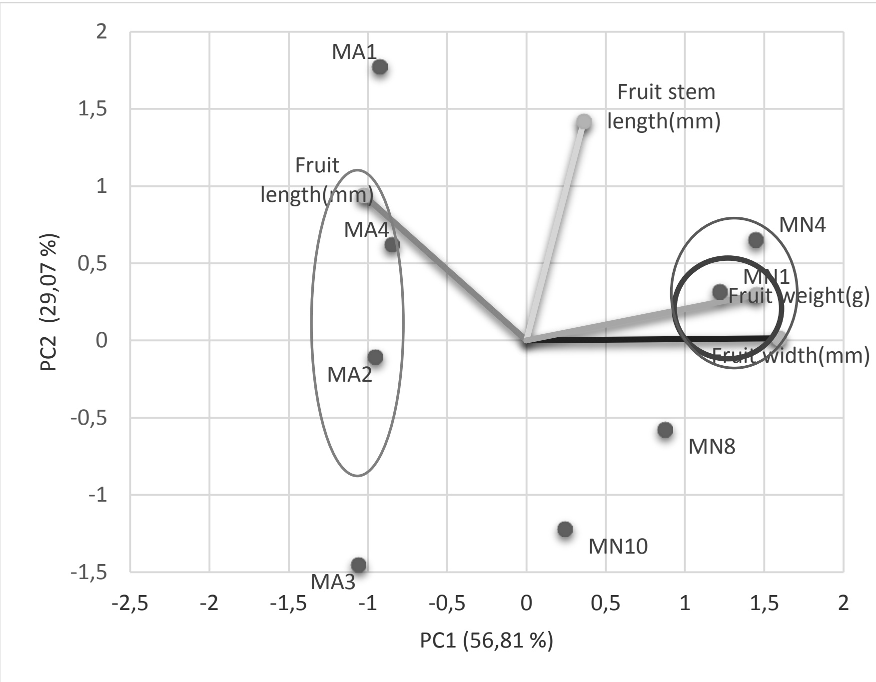


Figure 1: PCA analysis of morphological characteristics

On the biplot, 56.81% of genotype variability was explained with PC1, while 29.07% was explained by PC2 (Figure 1). The genotypes of black mulberry (MC1 and MC4) were isolated by PC1 analysis based on the pomological characteristics (fruit weight and width), while PC2 analysis grouped the genotypes of white mulberry based on fruit length (MA1, MA2, MA4). These data are in accordance with ANOVA (Table 1), where black mulberry genotypes stand out by fruit weight and width, and white mulberry genotypes stand out by fruit length.

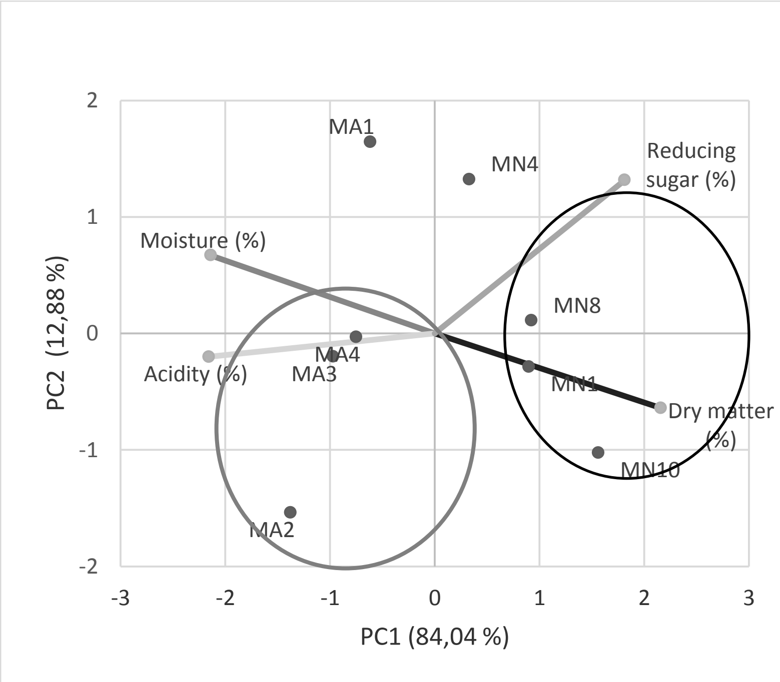


Figure 2: PCA analysis of chemical composition of fruit

PCA analysis of chemical composition of self-sown black and white mulberry revealed that: PC1 analysis showed that black mulberry samples stand out when it comes to dry matter and sugar contents (MN1, MN3 and MN4), while PC2 analysis showed that white mulberry samples stand out when it comes to acids content (MA1, MA2, MA4), which is in accordance with ANOVA (Table 2).

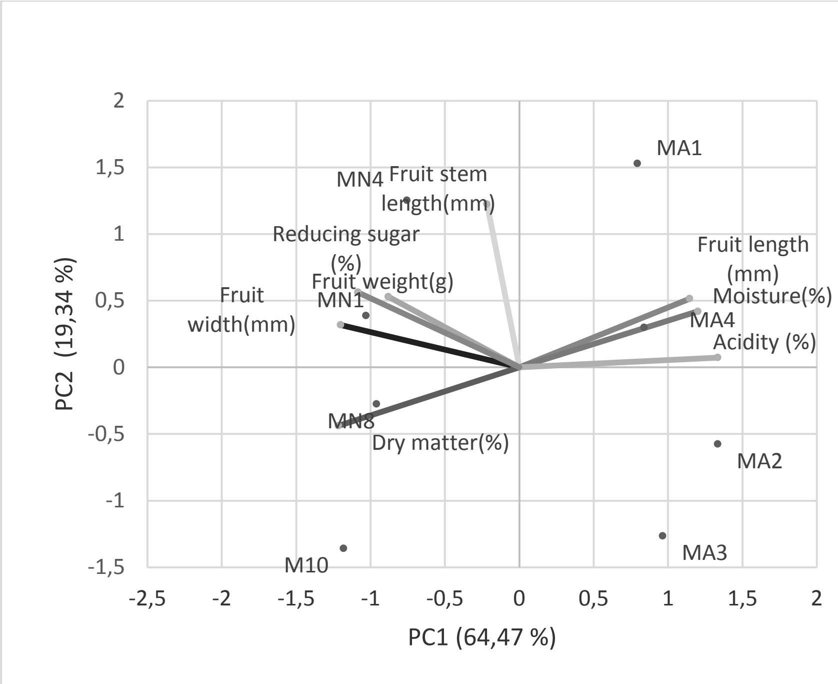


Figure 3: PCA analysis of morphological and chemical properties

Based on the biplot of PCA analysis of total results of pomological characteristics between the investigated genotypes of black and white self - sown mulberry (Figure 3), it can be concluded that black mulberry genotypes stand out when it comes to fruit weight and dry matter. White mulberry genotypes are grouped on the basis of total acids and fruit length. It can be concluded that these are the characteristics of black and white mulberry at the area of North - Western Bosnia.

CONCLUSIONS

The present study reveals that black and white mulberry genotypes have relatively high nutritional potential and wide variation was observed among genotypes in terms of nutrient contents. The variation of morphological and chemical–technological properties of self-sown genotypes of mulberry in North-Western Bosnia could be the result of heterozygote nature of seed propagated genotypes and the effect of different environmental conditions where the genotypes are grown. This study is also a step towards identification of this fruit as a potential healthy food, which may also be used in food industry and also have pharmaceutical interest. Based on the results obtained, mulberry fruits were found to serve as a potential source of food diet and natural antioxidants. The results on the composition of mulberry fruits might be of use to consumers and food technologists and provide valuable genetic resources for breeding programmes. The results of the study are helpful for understanding the variability and attempting the selection of superior desirable mulberry genotypes for bringing to commercial cultivation. These genotypes can be used for future breeding activities to obtain healthier mulberry fruits.

REFERENCES

Aljane, F. and Sdiri, N. (2016): Morphological, phytochemical and antioxidant

AOAC (1984): Official Methods of Analysis. Association of Official Analytical Chemists. 14th Edition, AOAC, Arlington.

Datta, R. K. (2000): Mulberry cultivation and utilization in India. FAO Electronic Conference on Mulberry for Animal Production.

Dimitrijević, D. S. (2014): Analiza hemijskog sastava i antioksidativne aktivnosti ekstrakta duda (*Morus* spp. *Moraceae*) Journal of Food and Nutrition Research. 53, 22-30.

Ercisli, S., i Orhan, E. (2007a): Chemical composition of white (*Morus alba*), red (*Morus rubra*) and black (*Morus nigra*) mulberry fruits. Food Chem. 103:1380- 1384

Ercisli, S. i Orhan, E. (2007b): Molecular Characterization of Mulberry Accessionsin Turkey by AFLP Markers. J. Amer. Soc. Hort. Sci. 133(4):593–597.

Ercisli, S. i Orhan, E. (2008): Some physico-chemical characteristic of black mulberry (*Morus nigra* L.) genotipes from Northeast Anatolia region of Turkey. Sci Hort. 116:41 - 46.

Ercisli, S., Tosun, M., Duralija, B., Voća, S., Sengul, M., Turad, M. (2010): Phytochemical content of some black (*Morus nigra* L.) and purple (*Morus rubra* L.) mulberry genotypes, FoodTecnol. Biotech., 48,102–106.

Eyduran, S. P., Ercisli, S., Akin, M., Beyhan, O., Gecer, M. K. Eyduran, E., Erturk, Y. E. (2015): Organic acids, sugars, vitamin C, antioxidant capacity, and phenolic compounds in fruits of white (*Morus alba* L.) and black (*Morus nigra* L.) mulberry genotypes. Journal of Applied Botany and Food Quality 88, 134-138.

Gecer, M., K., Akin, M., Gundogdu, M., Eyduran, S., P., Ercisli, S., Eyduran, E. (2016): Organic acids, sugars, phenolic compounds, and some horticultural characteristics of black and white mulberry accessions from Eastern Anatolia. Canadian Journal of Plant Science 96 (1): 27-33.

Gerasopoulos, D. and Stavraulakis, G. (1997): Quality characteristics of four mulberry (*Morus* spp) cultivars in the area of Chania Greece. J. Sci. Food Agricult. 73:261- 264.

Hammer, O.; D. A. T. Harper, P. D. Ryan. (2001): PAST: Paleontological Statistic software package for education and data analysis. Paleontologia Eletronica 4(1): 1-9. http://palaeo-electronica.org/2001_1/past/issue1_01.htm [Accessed: 04/IX/2017]

Holecyova, J., Brindza, J. and Balatova, Z. (2009): Biological and economical value of naturally spread population of black mulberry (Morus nigra L.) in Slovakia. Acta Hortic. 818, 109-114.

Imran, M., Khan, H., Shah, M., Khan, R., and Khan, F. (2010): Chemical composition and antioxidant activity of certain Morus species. J. Ahejiang Univ. Sci. B. 1112973–980. doi:10.1631/jzus.B1000173.

Koyuncu, F., Koyuncu, M. A., Yıldırım, F., Vural, E., (2004): Evaluation of Black Mulberry (*Morus nigra* L.) Genotypes from Lakes Region, Turkey Europ. J. Hort. Sci., 69 (3). S. 125–131.

Kutlu T., Durmaz G., Ates B., Yilmaz I., Cetin M. S. (2011): Antioxidant properties of different extracts of black mulberry (*Morus nigra* L.), Turk. J. Biol., 35, 103–110.

Kurtović, M., Gaši, F., Grahić, J., Maličević, A., Okić, A., Grbo, L. (2016): Jagodasto voće: biologija, tehnologija uzgoja, rasadnička proizvodnja i oplemenjivanje. Grafičar promet doo Sarajevo.

Mahmood, T., Anwar, F., Abbas, M., Boyce, M., Saari N. (2012): Compositional variation in sugars and organic Acids at Different Maturity Stages in Selected Small Fruits from Pakistan. *Int. J. Mol. Sci.* 2012, *13*(2), 1380-1392;

Mikulic-Petkovsek, M., Schmitzer, V., Slatnar, A., Stampar, F., and Veberic, R. (2012): Composition of sugars, organic acids and total phenolics in 25 wild or cultivated berry species. J. Food Sci. 77(10), 1064- 1070.

Milivojević, J., Nikolić, M., Dragišić-Maksimović, J. (2009): Pomološka i antioksidativna svojstva sorti jagode gajenih u regionu Mačve". Zbornik radova 24. Savetovanja o unapređenju proizvodnje voća i grožđa. Grocka 15 (5), 83-90.

Okatan, V., Polat, M., Askin, M., A. (2016): Some physiko – chemical characteristics of black mulberry (*Morus nigra* L.) in Bitlis. Scientific papers. Series B, Horticulture. Vol. LX, 27 – 30.

Ordom E. Z., Gomez J. D., Attuone M. A., Isla M. L. (2006): Antioxidant activities of Sechium edule (Jacq.) Swart extracts, Food Chem., 97, 452–458

Predojević, M., Ognjanov, V., Šavikin, K., Ljubojević, M., Bošnjaković D., Barać, G., Čukanović, J. (2012): Selekcija duda na osnovu pomoloških i biohemijskih karakteristika. 14. Kongres voćara i vinogradara Srbije sa međunarodnim učešćem, str 105., 9-12. okt 2012, Vrnjačka Banja. ISBN 978 – 86-7834-163-2.

Radojkovic, M. M., Zekovic, Z. P., Vidovic, S. S., Kocar, D. D., and Maškovic, P. Z. (2012): Free radical scavenging activity and total phenolic and flavonoid contents of mulberry (*Morus* spp. L., *Moraceae*) extracts. Hem. Ind. 664547–552.

Sanchez, E. M., Sanchez, A. C., Carbonell-Barrachina, A. A., Melgarejo, P., Hernandez, F., and Martinez-Nicolas, J. J. (2014): Physicochemical characterization of eight Spanish mulberry clones: Processing and fresh market aptitudes. Int. J. Food Sci. Technol. 49: 477 – 483.

Sass-Kiss A., Kiss J., Milotay P., Kerek M. M., Toth -Markus M. (2005): Differences in anthocyanin and carotenoid content of fruits and vegetables, Food Res. Int., 38, 1023–1029.

Singhal, B. K., Khan, M., A., Dhar, A., Baqual, F., M., Bindroo, B., B. (2010): Approaches to industrial exploatation of mulberry (Morus sp.) Fruits. Journal of Fruit and Ornamental Plant Research. Vol. 18 (1) 2010: 83-99.

Skender, A., Ajdinović, T., Bećirspahić, D. (2015): The comparison of phenotypic characteristics of current varieties and wild species of Fragaria. Genetika,Vol 47, No 1, 45-52.

Skender, A., Bećirspahić, D., Mujagić – Pašić, A., Joldić, S., Kulelija, B. (2014): Genofund of Native Fruit In the Una – Sana Canton. International Symposium on Sustainable Development «Biotechnology for Sustainable Development», Sarajevo, Bosnia and Herzegovina. Book of abstracts. Pp 140.

Stinzing, F. C., Stinzing, A. S., Carle, R., Frei, B., Wrolstad, R. E. (2002): Color and antioxidant properties of cyaniding - based anthocyanin pigments, J. Agric. Food Chem., 50, 2002, 6172 – 6181.

Yilmaz, K. U., Zengin, Y., Ercisli, S., Demirtas, M. N., Kan, T., Nazli, A. R. (2012): Morphological diversity on fruit characteristics among some selected mulberry from Turkey. The Journal of Animal & Plant Sciences, 22(1): 211-214.

Zadernowski, R., Naczk, M., Nesterowicz, J. (2005): Phenolic acid profiles in some small berries, J. Agric. Food Chem., 53, 2005, 2118 – 2124.

ANTHOCYANINS CONTENT IN BILBERRY (*Vaccinium myrtillus* L.) FRUITS FROM THREE POPULATIONS IN BOSNIA AND HERZEGOVINA*

SADRŽAJ ANTOCIJANINA U TRI POPULACIJE DIVLJE BOROVNICE (*Vaccinium myrtillus* L.) IZ BOSNE I HERCEGOVINE

Adnan Hodžić[1], Amila Vranac[1], Mirsad Kurtović[1], Jasmin Grahić[1], Senad Memić[1], Fuad Gaši[1]

Original scientific paper

Summary

The aim of the undertaken study was to investigate anthocyanins content of bilberry fruit, collected from three populations from north-eastern, eastern and central Bosnia and Herzegovina, during 2015 and 2016. Analysis of variance revealed that both the population and the season had a significant effect on the examined trait, as did the interaction of these factors. A *post-hoc* test only revealed a significant difference in anthocyanin content of bilberry fruit collected from north-eastern and eastern Bosnia and Herzegovina. A genetic analyses of all three populations as well as of their habitats, which is currently ongoing, might shed light on the results obtained in this study.

Key words: *Evaluation, berry fruit, geographical dispersion*

Rezime

Cilj istraživanja je bio ispitati sadržaj antocijanina u divljim borovnicama, uzetih iz tri populacije na području sjeveroistočne, istočne i centralne Bosne i Hercegovine, tokom 2015. i 2016. godine. Analizom varijanse je utvrđen značajan uticaj populacije, sezone i interakcija ovih faktora na ispitivani parametar. *Post hoc* testom je obznanjeno postojanje signifikantne razlike u sadržaju antocijanina između divljih borovnica sakupljenih sa područja svjeroistočne i istočne Bosne i Hercegovine. Genetske analize tri populacije divljih borovnica, kao i analize njihovih staništa, a koje su u toku, moći će dodatno pomoći u rasvjetljavanju rezultata dobijenih u ovom istraživanju.

Ključne riječi: *Evaluacija, borovnica, geografska rasprostranjenost*

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Faculty of Agriculture and Food Sciences, University of Sarajevo, Zmaja od Bosne 8, 71 000 Sarajevo, Bosnia and Herzegovina

INTRODUCTION

Bilberry (*Vaccinium myrtillus* L.) naturally grows at higher altitude on almost all mountains in Bosnia and Herzegovina (Kurtović *et al*., 2016). Notable populations of this species are located on the Konjuh mountain (north-eastern Bosnia), near Srebrenica (eastern Bosnia) and surrounding the town of Fojnica (central Bosnia and Herzegovina). In these regions, bilberry fruits are traditionally collected from natural populations and consumed either fresh or processed, while dried fruits and other parts of the plant are also traditionally used for medicinal purposes (Sarić-Kundalić *et al*., 2011). Bilberry represents one of the richest natural sources of anthocyanins, which among other things give the berry its specific colour. These bioactive components have a proven health-promoting and anti-inflammatory properties (Pibrger *et al*., 2011; Miyake *et al*., 2012) making them very attractive to consumers, as well as food and pharmaceutical industry (Johnson *et al*., 2010). Although total content of phenols and anthocyanins of bilberry fruits has been investigated among populations of this species in central Bosnia and Herzegovina (Rimpapa *et al*., 2007; Tahirovic *et al*., 2009), analysing geographically dispersed populations would allow for a more thorough evaluation. Therefore, the aim of the study was to investigate anthocyanins content of bilberry fruits collected from three populations from north-eastern, eastern and central Bosnia and Herzegovina, during 2015 and 2016.

MATERIALS AND METHODS

Bilberry fruits were collected, during 2015 and 2016, from three significant populations of this specie located on the Konjuh mountain (north-eastern Bosnia, Kladanj area), near Srebrenica (eastern Bosnia) and surrounding the town of Fojnica (central Bosnia and Herzegovina). The sampling was done using the following protocol: Within each population three plots with individual area of at least 30 square meters were marked, with the distance between plots of at least 15 meters, in order to reduce the risk of sampling identical clonal offspring. Five kilograms of ripe fruit (determined based on the berry colour) were harvested from the marked plot, mixed and divided into ten samples. Extraction of samples for anthocyanins analysis was carried out as follows: 10 grams of fruits were mixed with extraction solvent (methanol containing 3% formic acid and 1% BHT – for preventing the oxidation) and homogenized for 2 minutes using T-18 Ultra Turrax (IKA - Labortechnik, Staufen, Germany). The mixture was ultrasonificated in ultrasound ice bath (Elmasonic S 69 H; Elma Schmidbauer, Germany), and centrifugated at 10 000 rpm during 7 minutes at 0 °C (Thermo Scientific SL16 Centrifuge Series, San Jose, CA, USA). The obtained supernatants were filtered through Chromafil AO-45/25 polyamide filters (Macherey-Nagel, Düren, Germany) and used for analysis. Determination of the anthocyanins content in bilberry fruits was conducted using the pH differential method (Gusti and Wrolstad, 2001). Prior to measurements, optimal dillution factor and maximal apsorbance wavelenght were adjusted using the pH 1.0 buffer solution. Afterwards, sample extracts (0.2 mL) were separately mixed with both pH 1.0 and pH 4.5 buffer solutions, at optimal dillution rate (1:20). The apsorbances of samples were measured at 510 and 700 nm using UV-1700 spectrophotometer (Shimadzu, Japan). The obtained values were expressed as miligrams of cyanidin-3-glucoside per 100 grams of fruits fresh weight. An analyses of variance (Excel, MS Office 2013) was conducted on the obtained results, as well as the *post hoc* (Tukey) test ($p \leq 0.01$).

RESULTS AND DISCUSSION

The highest average values for total monomeric anthocyanin pigment content of bilberry fruit was measured among samples collected from Srebrenica (eastern Bosnia) in 2016, while the lowest content was detected among samples from Konjuh mountain (north-eastern Bosnia, Kladanj area) in 2015 (table 1). The difference in anthocyanins content between these population, in both seasons, was statistically significant ($p \leq 0.01$), while the difference for this parameter was not significant among other investigated populations. The effect of the season as well as the effect of the interaction of a specific population and season, on the anthocyanins content, displayed statistical significance, while the *post hoc* test determined that overall the anthocyanins content was significantly higher in 2016 compared to 2015 (table 1 and figure 1). The differences in anthocyanins content in observed bilberry populations are highly affected by habitate and climate characteristics (altitude, sunlight exposure, etc.), since the biosynthesis of these pigments is directly dependent on the received amount of sunlight.

Table 1. Anthocyanins content (mg $100g^{-1}$ FW) in bilberries from three populations in B&H during 2015 and 2016

Location	2015	2016
Fojnica	401.3 ± 19.0 ab	439.4 ± 4.28 ab
Kladanj	380.1 ± 17.5 b	437.2 ± 6.16 b
Srebrenica	422.0 ± 22.7 a	448.8 ± 3.52 a

a-c - different letters indicate statistically significant differences between locations in the same year at $p \leq 0.01$.

In this study, anthocyanins content values for the three B&H bilberry ranged between 380-449 mg 100 g^{-1} FW. The values obtained here are in accordance comparable to the data reported by Latti *et al*. (2008) on the Finnish bilberry populations (350-525 mg 100 g^{-1} FW), but higher then what was reported (330-344 mg 100 g^{-1} FW) for Italian populations (Giovanelli and Buratti, 2009).

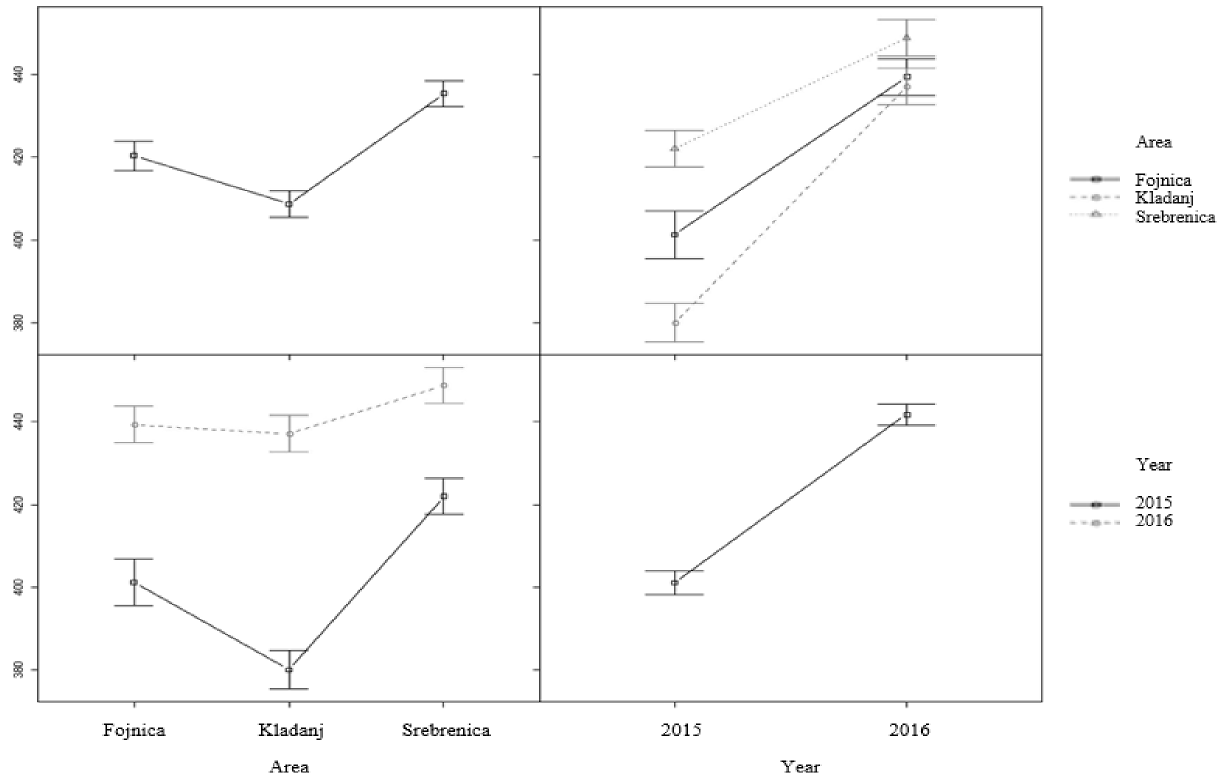


Figure 1. Results of *post hoc* test conducted on the anthocyanins content (mg 100g^{-1} FW) of three bilberry populations in Bosnia and Herzegovina, measured during two consecutive seasons

CONCLUSIONS

The seasonal influence on the total monomeric anthocyanin pigment content of bilberry fruit was significant, as is presumably the influence of other environmental factors specific to each population. However, a currently ongoing genetic analyses of all three populations might shed additional light on the results obtained in this study, especially considering that aside from the environmental factors, genetic predisposition has a significant influence on the anthocyanin content in bilberry (Prior *et al.*, 1998; Kalt *et al.*, 2000). It is also worth noting that the obtained values are rather high, indicating that the bilberry populations present in B&H are of great value.

REFERENCES

Giovanelli, G., Buratti, S. (2009): Comparison of polyphenolic composition and antioxidant activity of wild Italian blueberries and some cultivated varieties. Food Chemistry, 112: 903-908.

Gusti, M. M., Wrolstad, R. E. (2001): Unit F1.2.1-13. Anthocyanins, Characterization and measurement with UV visible spectroscopy. In: Wrolstad, R.E. (Edt), Current protocols in food analytical chemistry. Wiley, New York, USA.

Johnson, B. J., Lin, B., Bongard, J. E. (2010): Genus Vaccinium: medicine, cosmetics, and coatings. Recent Patents on Biotechnology, 4: 112-124.

Kalt, W., McDonald, J. E., Donner, H. (2000): Anthocyanins, phenolics, and antioxidant capacity of processed lowbush blueberry products. Journal of Food Science, 65: 390-393.

Kurtović, M., Gaši, F., Grahić, J., Maličević, A., Okić, A., Grbo, L. (2016): Jagodasto voće: biologija, tehnologija uzgoja, rasadnička proizvodnja i oplemenjivanje. Grafičar promet d.o.o. Sarajevo.

Latti, A. K., Riihinen, K. R., Kainulainen, P. S. (2008): Analysis of anthocyanin variation in wild populations of bilberry (*Vaccinium myrtillus* L.) in Finland. J. Agric. Food Chem., 56: 190-196.

Miyake, S., Takahashi, N., Sasaki, M., Kobayashi, S., Tsubota, K., Ozawa, Y. (2012): Vision preservation during retinal inflammation by anthocyanin-rich bilberry extract: cellular and molecular mechanism. Laboratory Investigation, 92: 102–109.

Piberger, H., Oehme, A., Hofmann, C., Dreiseitel, A., Sand, P. G., Obermeier, F., Schoelmerich, J., Schreier, P., Krammer, G., Rogler, G. (2011): Bilberries and their anthocyanins ameliorate experimental colitis. Molecular Nutrition and Food Research, 55: 1724–1729.

Prior, R. L., Cao, G., Martin, A., Sofic, E., McEwan, J., O’Brien, C., Lischner, N., Ehlenfeldt, M., Kalt, W., Krewer, G., Mainland, C. M. (1998): Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity and variety of Vaccinium species. J. Agric. Food Chem., 46: 2686-2693.

Rimpapa, Z., Toromanović, J., Tahirović, I., Šapčanin, A., Sofić, E. (2007): Total content of phenols and anthocyanins in edible fruits from Bosnia", Bosnian Journal of Basic Medical Sciences, 7, 2: 119-122.

Sarić-Kundalić, B., Dobeš, C., Klatte-Asselmeyer, V., Saukel, J. (2011): Ethnobotanical survey of traditionally used plants in human therapy of east, north and north-east Bosnia and Herzegovina. Journal of Ethnopharmacology, 133: 1051-1076.

Tahirovic, I., Toromanovic, J., Sapcanin, A., Hrvat, A., Sofic E. (2009): Antioxidant capacity against peroxyl free radicals of various edible fruits from Bosnia. Planta Med. DOI: 10.1055/s-0029-1234520

THE EFFECT OF PRUNING ON FRUITING CAPACITY OF BLACK MAGIC TABLE GRAPE VARIETY*

UTICAJ REZIDBE NA RODNOST STONE SORTE VINOVE LOZE BLACK MAGIC

Mersija Delić[1]**, Fikreta Behmen[1], Semira Sefo[2], Pakeza Drkenda[1], Violeta Dimovska[3], Amer Sunulahpašić[4]

Original scientific paper

Summary

Black Magic table grape variety is a newly introduced variety in Bosnia and Herzegovina. The aim of this paper was to determine the effect of various types of pruning on fruiting of Black Magic variety in the conditions of Herzegovina, given that no researches have been done so far for this variety. The research lasted three years (2011, 2012 and 2013), and three pruning variants were used (28, 32 and 40 buds/vine). On the basis of obtained results it is evident that the values of the examined parameters were the highest in 2011 (grape yield /vine of 11.06 kg, total number of productive canes/vine was 24.72, number of productive canes/vine 23.79 and number of bunch/vine 44.87), and the lowest in 2013 (grape yield /vine 7.49 kg, total number of productive canes/vine 21.02, number of productive canes/vine 21.11 and number of bunch/vine 23.16). Pruning variant III (40 buds/vine) had the highest levels of these parameters in all three experimental years, and variant I (28 buds/vine) the lowest.

Key words: *Black Magic, table grapevine, pruning, yield.*

Rezime

Stona sorta grožđa Black Magic je novointrodukovana sorta u Bosni i Hercegovini. Cilj ovog rada je bio da se utvrdi uticaj različitih načina rezidbe na rodnost sorte Black Magic u uslovima Hercegovine, obzirom da nisu rađena istraživanja na ovoj sorti. Istraživanja su trajala tri godine (2011, 2012 i 2013), a primijenjene su tri varijante rezidbe (28, 32 i 40 okaca/čokotu). Na osnovu dobijenih rezultata može se vidjeti da

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
**Izvod iz doktorske disertacije
[1] University of Sarajevo, Faculty of Agricultural and Food Sciences, Department of Plant Production, Bosnia and Herzegovina
[2] University "Džemal Bijedić" of Mostar, Agromediterian Faculty, Bosnia and Herzegovina
[3] Goce Delčev University, Faculty of Agriculture, Department of Plant Production, Republic of Macedonia
[4] Ministry of Agriculture, Water management and Forestry of Central Bosnia Canton, Bosnia and Herzegovina
**Corresponding author: mersija.delic@gmail.com, m.delic@ppf.unsa.ba

su vrijednosti ispitivanih parametara bile najveće 2011. godine (prinos grožđa/čokotu 11,06 kg, ukupan broj lastara/čokotu 24,72, broj rodnih lastara/čokotu 23,79 i broj grozdova/čokotu 44,87), a najmanje 2013. godine (prinos grožđa/čokotu 7,49 kg, ukupan broj lastara/čokotu 21,02, broj rodnih lastara/čokotu 21,11 i broj grozdova/čokotu 23,16). Varijanta rezidbe III (40 okaca/čokotu) imala je najveće vrijednosti navedenih parametara tokom sve tri ogledne godine, a varijanta I (28 okaca/čokotu) najmanje.

Ključne riječi: *Black Magic, stono grožđe, rezidba, prinos*

INTRODUCTION

Table grapes is one of the most important crops in the world and special attention is paid to improving the yield and quality of fruit (Bruhn *et al.*, 1991). A large number of newly created table varieties of various biological and economic – technological characteristics have been introduced in the area of Herzegovina in recent years. So far, these varieties have not been studied from the standpoint of their adaptation to the environmental conditions of Herzegovina. Therefore, the subject of this paper was to study in detail the effect of pruning on fruiting capacity of Black Magic table grape variety, being one of a large number of newly introduced table grape varieties, in the conditions of Herzegovina.

Black Magic table grape variety was created at the Institute of Viticulture in Chisinau, Moldova, where it was named Codreanca. It was introduced to Italy through Vivai Cooperativi Rauscedo and they have the exclusivity for this variety. The variety was renamed to Black Magic and that is its international name. It was created by crossing Moldova x Marshal (origin *V. labrusca*) varieties (Nicolaescu, 2009; Pizzuto, 2013).

Black Magic is a table variety with seeds. Fruiting capacity is good, fruiting coefficient is 1.19. Its yield is quite high and ranges between 15 to 20 t/ha. Budding period is in the third decade of March, and ripening in the third decade of July which classifies it as a very early variety. Vine is vigorous and five-lobed leaves are of medium size. Bunches are medium to large in size, conical pyramid in shape, with an average weight of 400 – 500 g. The berry is medium-sized, egg-shaped, and its skin is dark purple and covered with abundant bloom. The sugar content in the grape juice is 17.4 %, and total acids is 5.2 g/l. Resistance to transportation is good (www.vivairauscedo.com).

METHODS

Field studies were conducted at the facility "Vinogradi" doo Mostar, and laboratory ones at the Faculty of Agriculture and Food Sciences in Sarajevo. Research lasted three years (2011, 2012, and 2013). In the studied vineyard, experiment was set up by the method of random selection, and health status and uniformity of vegetative

potential of vines were good. This vineyard was planted in 2008, with vine spacing 3m x 1.2 m, with Moser cordon as the training system. Three variants of pruning were studied on Black Magic table grape variety (28, 32, and 40 buds per vine) (table 1). The experiment was set up on 72 vines in four repetitions (6 vines per repetition). Black Magic was grafted on Paulsen 1103 rootstock. In the vineyard there is a system of drip irrigation. On the experimental location, this variety is in intensive production with regular application of agricultural practices.
During the examination, monitored were the beginning, duration and completion of individual phases of the phenological development of vine including: bleeding, beginning of bud swelling and formation of canes, flowering and fertilization, development of green berries to the emergence of veraison, grape ripening and maturation of canes, and leaf-fall. Dates of the beginning of dormancy and vegetation periods as well as individual phases during the vegetation period were recorded for all three experimental years, and mean dates of the beginning of certain periods and phases of the development of vine during the year were determined using the method of arithmetic mean. The phenological development of varieties was monitored by the method of Lazarevski.

The experiment included the following variants of pruning:

Table 1. Pruning variants with the specified number of buds

Variant	Pruning	Number of short sprouts	Number of long sprouts	Number of buds per vine
Variant I	short	4(5)+ 4(2)	0	28
Variant II	mixed	4 (2)	4(6)	32
Variant III	mixed	4 (2)	4 (8)	40

During the research, the following indicators were examined:
1. Phenological development of the variety,
2. Fruiting capacity of canes,
3. Weight of grapes per vine,
4. Number of bunch per vine.

Fruiting capacity of the examined varieties was determined and expressed per vine, per variant and per repetition.
Fruiting capacity of the variety was determined on the basis of the following indicators:

- Number of developed canes,
- Number of fruiting canes,
- Number of bunch per vine,
- Weight of grapes per vine (g).

The parameters obtained during the study were processed in the SPSS software package.

Environmental conditions

Anticipated changes in the climate of the European winegrowing regions in the following decades can significantly alter both the range and distribution of grape varieties that are currently in use (Chiriac, 2007). Due to the higher temperatures, traditional limitations to winegrowing are changing as well as phenological aspect, production and quality of grapes, as it happened in Western Europe, mainly in France, in the past 15 years (Enache, 2008).

In determining the suitability of conditions for growing vines in an area, climate is the crucial factor. If climatic conditions are not suitable for a certain variety, major changes occur in the duration and passage through phenophases of development which is reflected in the yield and quality of grapes.

Crucial impact on the climate of Mostar vineyard region has its openness towards the sea, along the valley of the river Neretva, and the separation from the northern areas by mountain ranges. To analyze climatic conditions we used data of the meteorological station Mostar obtained through the FBiH Hydro-meteorological Institute.

Table 2. Basic hydro-meteorological factors in Mostar winegrowing region

Indicator	1961 - 1990	2011	2012	2013
Mean annual air temperature (°C)	14.1	16.2	16.1	15.9
Mean vegetative air temperature (°C)	18.83	21.76	22.1	21.16
Absolute minimum air temperature (°C)	-14.2	-1.6	-7.4	-2.4
Absolute maximum air temperature (°C)	41	40.4	41.8	41.1
Precipitation during vegetation (mm)	518	396.5	744.4	931.1
Annual precipitation (mm)	1102	872.5	1394.9	2188.3
Length of the vegetation period (days)	239	235	236	237
Length of insolation (hours)	2287	2629.9	2656.9	2464.2

Climatic conditions in the years of research deviated from long-term averages according to both the recorded air temperatures and amount of precipitation. During the study years, air temperature was considerably higher compared to a multiannual average. The amount of rainfall was also higher compared to the multiannual average, except in 2011 (table 2).

RESULTS AND DISCUSSION

Phenology is usually described as the art of observing the life cycle phases or the activities of plants and animals in the periodicities of their occurrence throughout the year. Phenology is a scientific field existing on the border between floristics, ecology and meteorology, especially agro meteorology (Lieth, 1970). The length of periods between phenological phases varies considerably depending on the variety of grape, climate, and geographical location (Jones and Davis, 2000).

Table 3. Dates of occurrence of phenophases in the development of Black Magic variety within the annual cycle of vine development in agro-ecological conditions of the Vrapčići – Mostar site

Year of observa-tion	Phenophases of development						
	Bleeding	Bud break	Flowering		Veraison	Full ripeness	End of vegetation
			beginning	end			
2011	19.03.	01.04.	20.05.	30.05.	04.07.	25.07.	08.11.
2012	20.03	03.04.	26.05.	04.06.	10.07.	01.08.	11.11.
2013	20.03.	04.04.	27.05.	06.06.	11.07.	03.08.	12.11.
Average	20.03.	03.04.	24.05.	03.06.	08.07.	30.07.	10.11.

Phenophases of development in Black Magic variety took place in accordance with the temperature conditions in the years of research. The duration of all phenophases of development was within the time lines typical of this region (table 3).

Fruiting capacity of the variety

Fruiting capacity as economic characteristic is a very important factor in the production cost-effectiveness. It is expressed as grapes yield per square unit, where the yield, in addition to the generative potential as a varietal property, is largely influenced by environmental conditions and applied technology (Maletić *et al.*, 2008). As a rule, in table grape varieties, the first 2 to 3 (or even 4-5) buds on a fruiting cane are very little or not at all fruitful. Therefore, fruiting capacity of the buds or canes in the vegetation begins to manifest from the third or the fourth and fifth bud upward, so it is the basic and sole reason why mixed or long pruning is applied in table grape varieties (Tadijanović, 1993).

Analysis of variance shows that the observed parameters of fruiting capacity of Black Magic variety were statistically significantly influenced by both experimental factors (pruning variant and year), except for the parameter of grapes yield where the pruning variant had no statistically significant effect. There were no interactions between experimental factors (table 4).

Table 4. Average values of grape fruiting parameters in the years of research

Parameter		Pruning variant	Year of research			Average for the pruning variant
			2011	2012	2013	
Total number of canes per vine		I II III	23.46 24.08 26.63	18.92 20.92 24.71	20.53 19.86 22.67	20.97[b] 21.62[b] 24.67[a]
	Average for the year		24.72[a]	21.52[b]	21.02[b]	
Number of fruiting canes		I II III	22.42 23.25 25.71	18.75 20.75 24.42	20.00 19.79 23.54	20.39[b] 21.27[b] 24.56[a]
	Average for the year		23.79[a]	21.31[b]	21.11[b]	
Grape yield per vine		I II III	10.20 10.58 12.38	10.14 10.38 11.14	7.36 7.39 7.73	9.23[ns] 9.45[ns] 10.42[ns]
	Average for the year		11.06[a]	10.55[a]	7.49[b]	
Number of bunch per vine		I II III	39.96 42.68 51.96	29.34 25.18 29.83	22.84 22.50 24.15	30.71[ab] 30.12[b] 35.31[a]
	Average for the year		44.87[a]	28.12[b]	23.16[c]	

In total number of canes per vine there was a statistically significant difference in 2011 (24.72) compared to 2012 (21.52) and 2013 (21.02). Also, there was a statistically significant difference between the pruning variants, so the Variant III (24.67) had a statistically significantly higher number of total canes compared to the other two pruning variants.

Number of fruiting canes per vine was in line with the relations identified in the analysis of the total number of canes per vine.

The yield of grapes per vine was statistically significantly higher in 2011 (11.06 kg) and 2012 (10.55 kg) compared to 2013 (7.49 kg).

The number of clusters per vine was statistically significantly different during all three years of research. Statistically significantly highest average values of the observed parameter were recorded in 2011 (44.87), then in 2012 (28.12), and the lowest in 2013 (23.16). When it comes to the variants of pruning, it can be stated that there is a statistically significant difference in the number of clusters per vine as well as the variant of pruning. Variant III (35.31) had a statistically significantly larger number of clusters compared to the variant II (30.12), but there was no statistically significant difference in the value of the observed parameter for the variant I (30.71) compared to the variants II and III.

The yield of grapes per vine in Black Magic variety amounted to 5.6 kg in the Tikveš winegrowing area (Dimovska *et al.*, 2013), and to 5.67 kg in conditions of Moldova

(Nicolaescu *et al*., 2009). In this study, the yield of grapes per vine was significantly higher compared to the studies of the aforementioned authors. This difference in the yield of grapes can be explained by the fact that the fruiting bud load of a vine in these authors was significantly lower (20 buds/vine), while in this experiment it was far higher (28.32 and 40).

CONCLUSIONS

1. Agro-ecological conditions of the site were presented using climate parameters for a multi-year sequence from 1961 to 1990, as well as the three experimental years (air temperature, precipitation, cloudiness) and soil parameters. Climatic conditions in the years of research deviated from the standard average in terms of both air temperature and amount of precipitation. The mean annual air temperature in the years of research was higher compared to the standard average by approximately 2°C and the average vegetation temperature by approximately 3°C. All three experimental years had extremely hot summers. Distribution of precipitation in the researched period was uneven, so during three years we had a number of months with extremely low or high levels of precipitation compared to multi – year average.

2. Phenophases of development in Black Magic variety took place in accordance with temperature conditions in the years of research. The duration of all phases of development was within the timelines typical of this region.

3. Fruiting capacity of the examined variety was shown using the following indicators:

    a. The total number of developed canes per vine was the largest in pruning variant III, which was to be expected given that the variant III had the highest number of remaining buds per vine.
    b. The number of fruiting canes was also the largest in the variant III, and the value of this parameter was statistically significantly influenced by both experimental factors (pruning variant and year).
    c. The yield of grapes per vine in the variant III was higher compared to other variants of pruning in the examined variety, where the yield of grapes per vine in 2011 and 2012 was higher compared to 2013.
    d. The number of bunch per vine in the variant III was the highest relative to other pruning variants, while the number of clusters per vine was the largest in 2011.

REFERENCES

Bruhn, C. M., Feldman, N., Garlitz, C., Harwood, J., Ivans, E., Marshall, M., Riley, A., and Williamson, M. (1991): Consumer perceptions of quality: Apricots, cantaloupes, peaches, pears, strawberries, and tomatoes. J. Food Qual. 14:187–195.

Chiriac, C. (2007): Influence of climate change upon environment in the Cotnari Vineyard, Annals of Stephan the Great University, Romania, XVI, http://www.atlas.usv.ro/www/anale/datagrid.

Dimovska Violeta, Beleski, K., Boskov, K., Ivanova Violeta, Ilieva Fidanka (2013): The productive characteristics on Black Magic table grape variety, growing in the Tikves's vineyard, republic of Macedonia. IV International Symposium "Agrosym 2013", pp. 141-146. Jahorina, B&H.

Enache, V., Simion C., Donici A. and Popescu A. (2008). Aspects of the Desertification risk in the South of Moldova, Romania, Conference on Desertification, University of Ghent, Belgium, 23 January 2008.

Jones, V. G., Davis, E. R., (2000): Climate influences on grapevine phenology, grape composition, and wine production and quality for Bordeaux, France. Am. J. Enol. Vitic., Vol. 51, N_0 . 3., p. 249-261.

Lieth, H. (1970): Phenology in Productivity. Studies Analysis of Temperate Forest Ecosystems Ecological Studies, Volume 1, pp 29- 46.

Maletić E., Karoglan Kontić, J., Pejić, I. (2008): Vinova loza-ampelografija, ekologija, oplemenjivanje. Školska knjiga, Zagreb.

Nicolaescu, Gh., Derendovskaia Antonina, Perstniov, N., Stirbu, A., Tcaciuc Olga, Nicolaescu Ana, Ciobanu Tudor, Josan Silvia (2009): Gibberellin – as a determinant factor of grapes quality of Codreanca (Black Magic) variety. Lucrări ştiinţifice USAMUB, Seria B, vol LIII, pp 573 – 576.

Pizzuto, M. (2013): Evolució varietal en el món Clons, La genética a disposició dels objectiues de l'enóleg, XXIV Congrés de l' ACE, Italy.

Tadijanović, Đ., (1993): Oblici čokota i rezidba sa planiranjem prinosa vinove loze, Nolit – Beograd.

THE EFFECT OF CYCOCEL ON MORPHOLOGICAL CHARACTERISTICS OF ALPINE FORGET-ME-NOT (*Myosotis alpestris* F.W. Schmidt)*

UTICAJ CYCOCEL-a NA MORFOLOŠKE KARAKTERISTIKE PLANINSKE POTOČNICE (*Myosotis alpestris* F.W. Schmidt)

Jasna Avdić[1], Nermina Sarajlić[2]

Original scientific paper

Summary

Alpine forget-me-not (*Myosotis alpestris* F.W. Schmidt) is a very popular flowering plant for flower beds in public green areas, because of its exceptional aesthetical characteristics and early flowering. It is produced by sowing seeds in greenhouses, during summer and autumn, when, because of very changeable light intensity and high temperatures, young plants tend to elongate, which reduces their aesthetic value and at the same time makes handling and transport difficult. The aim of this paper is to investigate the effect of plant growth retardant Cycocel, commercially available as AgriChem CCC 750, to the height and the number of leaves and flower buds in forget-me-not. Plants were treated with 0,10% and 0,30% water solution, 15 days after replanting. Control group of plants was treated with water. The results showed that AgriChem CCC 750 caused significantly slower growth in young plants. It also affected the number and size of leaves and inflorescences, as well as the flower color intensity in treated plants.

Key words: *Myosotis alpestris, growth retardant, CCC, slower plant growth, morphological characteristics*

Rezime

Planinska potočnica (*Myosotis alpestris* F.W. Schmidt) je česta dvogodišnja ukrasna vrsta koja se zbog izrazitih estetskih osobina i ranog cvjetanja koristi za sadnju u cvjetne gredice na javnim zelenim površinama. Proizvodi se sjetvom sjemena u zaštićenim prostorima, u ljetnjem i jesenskom periodu, kada zbog promjenljivog intenziteta svjetla i visokih temperatura dolazi do pojave izduživanja biljaka, što smanjuje njihovu dekorativnost, a ujedno otežava rukovanje i transport. Cilj ovog rada

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Faculty of Agricultural and Food Sciences, University of Sarajevo, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina
[2] Faculty of Natural Sciences and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia
Correspondence: Prof. dr. Jasna Avdić, Faculty of Agricultural and Food Sciences, University of Sarajevo, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina jasnaa95@yahoo.de

bio je da se ispita djelovanje retardanta rasta Cycocel-a, tvorničkog naziva AgriChem CCC 750 na visinu, broj listova i broj cvasti planinske potočnice. Za tretiranje biljaka korišteni su vodeni rastvori koncentracije 0,10% i 0,30%, kojima su biljke zalijevane 15 dana nakon presađivanja. Kontrolna grupa biljaka je zalijevana čistom vodom.
Rezultati su pokazali da AgriChem CCC 750 znatno usporava rast mladih biljaka, utiče na promjenu broja listova i cvasti, ali i na intenzitet boje cvijeta.

Ključne riječi: *Myosotis alpestris, retardant rasta, CCC, usporeni rast biljaka, morfološke osobine*

INTRODUCTION

Myosotis alpestris F.W. Schmidt (Alpine Forget-me-not) is a popular biennial flowering plant, used for planting in flowerbeds and flowerpots in sunny or semi-shadowed locations. It is propagated by seed, which can be sown directly outside during summer, but is more often sown in greenhouses and glasshouses, resulting in development of better quality plants. The plants are planted in the desired location, where they overwinter and bloom in early spring next year (Đurovka *et al*., 2006). The seedlings grown in greenhouses tend to express the shade avoidance syndrome, elongating in order to avoid the shade of another plant. This makes the production process more difficult and decreases the ornamental value of individual plants. The plant growth can be controlled by growth regulators, which slow the plant growth, resulting in shorter and more compact seedlings, easier for handling and transport. The growth regulator's effectiveness depends on the manner and time of application and the plant species (Rademacher, 1995). One of the most commonly used plant growth retardants is Cycocel (chlormequat chloride, CCC), which blocks the synthesis of gibberellins (Cleland and Briggs, 1969). In the production of ornamental plants, Cycocel is used to reduce height in order to achieve more attractive, compact plants. The aim of this paper was to examine the effect of Cycocel, sold under the name AgriChem CCC 750, on the height, number of leaves and number of inflorescences in *Myosotis alpestris* plants.

MATERIALS AND METHODS

The research was carried out in the greenhouse of Public Communal Enterprise "Park" - Neđarići in Sarajevo. The seed used in the experiment was produced by Semenarna from Ljubljana (Slovenia). The seed was manually sown in wooden 40 × 30 cm boxes, in Florabella substrate, produced by Klassmann-Deilmann GmBH, based on slightly decomposed white peat with added black peat and lime, pH 5.5 to 6.5, fertilized by mineral fertilizers in N:P:K = 14:16:18 ratio. In a 3-4 leaf stage, the seedlings were transplanted in black polyethylene 2 dl pots, in the same substrate, mixed with river sand in 3:1 ratio, in order to improve its water-air regime. 15 days after transplantation, the plants were treated by 0,10%, 0,15% and 0,30% aqueous solution of AgriChem CCC 750, manufactured by AgriChem (Oosterhout, The Netherlands). The control group of plants was treated with water. Each group was represented by 40 plants. During the vegetation period, the height, the number of leaves and the number of flowers were measured. The results were analyzed by ANOVA Single factor, and if the F value was found to be significant ($P < 0.05$), the mean differences between the groups were tested by the Tukey HSD test, using MS Excel 2007 and XLSTAT 2010.

RESULTS AND DISCUSSION

The results of the study of the effect of AgriChem CCC 750 on the height, number of leaves and number of inflorescences of *Myosotis alpestris* are presented in table 1.

Table 1. The average heigth, number of leaves and number of inflorescences of *Myosotis alpestris* F.W. Schmidt plants treated with different concentrations of CCC

	0%	**0,10%**	**0,15%**	**0,30%**
Height	84,2 ± 6,11 a	70,15 ± 7,77 b	60,63 ± 9,11 c	50,47 ± 5,85 d
No. of leaves	50,05 ± 4,25 a	49,37 ± 4,52 a	40,67 ± 3,24 b	33,62 ± 2,29 c
No. of inflorescences	22,45 ± 2,57 a	21,1 ± 1,35 a	16,87 ± 1,78 b	13,85 ± 1,62 c

All treated plants were shorter than the plants from the control group. Even the smallest concentration of AgriChem CCC 750 (0,10%) caused the significant reduction in plant height, but the effect was more prominent in plants treated with higher concentrations. Similar results were obtained by El-Mokadem & Hadia (2008), who showed that the maximum concentration of 0,40% CCC caused the shortest stems on *Encelia farinosa* Torr. & A. Gray, by Pinto *et al*. (2005) in the experiment with *Zinnia elegans* Jacq. and Sarajlić & Avdić (2010) in the experiment with *Viola* × *wittrockiana* Gams.

The CCC treatment caused the reduction in number of leaves in all treated plants. The smaller number of leaves in treated plants was also reported by Bhattacharjee & Gupta (1981) for *Helianthus annuus* L. Rusch *et al*. (1987) for *Hydrangea macrophylla* (Thunb.) Ser., Auda *et al*., (2002) for *Barleria cristata* L. and El-Mokadem & Hadia (2008) for *Encelia farinosa*. The different results, with the increase in number of leaves after treatment with CCC were recorded for *Pelargonium* × *hortorum* L.H. Bailey by Welander (1984), *Zinnia elegans* by Pinto *et al*. (2005) and *Viola* × *wittrockiana* by Sarajlić & Avdić (2010).

Figure 1. The effect of Cycocel on *Myosotis alpestris* plants

The treatment with Cycocel also caused the reduction in number of inflorescences in *Myosotis alpestris* (Figure 1). The earliest formation of inflorescences was registered in plants treated with 0,15% solution of Agrichem CCC 750, and the latest formation was observed in plants treated 0,30% solution. According to Zeevaart (1964), the treatment with Cycocel led to significant reduction in number of flowers of *Pharbitis nil* (L.) Choisy plants. In *Viola* × *wittrockiana*, the largest number of flowers was recorded in plants treated with 0,10% CCC (Sarajlić & Avdić, 2010). Slower development and smaller number of flower buds was also registered by Anderson & Hartley (1990) in *Clarkia amoena* (Lehm.) Nels, and Ali & Elkiey (1995) in *Zantedeschia rehmannii* (L.) Spreng. On the other hand, Abou-Zied & Bakry (1978) reported earlier flowering and larger number of flowers in treated *Primula obconica* Hance plants, as did Tripathi *et al*. (2003) in *Tagetes erecta* L. and Matsoukis & Chronopoulou-Sereli (2005) in *Lantana camara* L.
It was also observed that *Myosotis alpestris* plants treated with 0,10% solution of AgriChem CCC 750 had more intensive flower color than the plants from the control group. The change in color was not noticed in plants treated with 0,15% and 0,30% solutions.

CONCLUSIONS

After the research of the effect of CCC growth retardant on *Myosotis alpestris* plants, the following conclusions can be made:

- Cycocel showed the significant effect on reduction of plant height, number of leaves and number of inflorescences. The higher concentrations had the stronger effect on all investigated characteristics.
- The plants treated with 0,10% solution were compact and had numerous inflorescences with more intensive flower color, so this concentration would be recommended for production of *Myosotis alpestris* plants.

- On the other hand, 0,30% CCC solution causes the shortest plants with smallest number of inflorescences and latest blooming, so this concentration is to be avoided in the production of this flowering plant species.

REFERENCES

Ali Y. S., Elkiey T. (1995): Effect of chlormequat and GA3 on growth and flowering of calla (*Zantedeschia rehmannii*). *Journal of King Saud University*. Agricultural Sciences. 7 (2): 271-282.

Anderson, R. G., Hartley, G. (1990): Use of growth retardants on satin flower, *Godetia*, for pot plant production. *Acta Hort*. (ISHS) 272: 285-292.

Abou-Zied E. N., Bakry M. Y. (1978): The effects of GA_3, CCC and B-9 upon growth, development and organic compounds in Primula obconica. *Scientia Horticulturae*, 9 (2): 175-180.

Đurovka M., Lazić B., Bajkin A., Potkornjak A., Marković V, Ilin Ž., Todorović V. (2006): Proizvodnja povrća i cveća u zatvorenom prostoru, Poljoprivredni fakultet, Novi Sad. pp. 161-163.

Sarajlić N., Avdić J. (2010): Uticaj Cycocel-a na morfološke osobine maćuhice (Viola × witrockiana Gams). XXI Naučno-stručna konferencija poljoprivredne i prehrambene industrije, Neum 2010, Zbornik radova: 371 – 377.

Cleland C. F., Briggs W. S. (1969): Gibberellin and CCC Effects on Flowering and Growth in the Long-day Plant *Lemna gibba*. *Plant Physiology* 44: 503-507.

Matsoukis A., Chronopoulou-Sereli, A. (2005): Interaction of chlormequat chloride and photosynthetic photon flux on the growth and flowering of *Lantana camara* subsp. *camara*. *Acta Hort*. (ISHS) 683: 265-270.

Pinto, A. C. R., Rodrigues, T. J. D., Leite, I. C. and Barbosa, J. C. (2005): Effect of daminozide, paclobutrazol and chlormequat on development and quality of potted 'Persian Carpet' zinnia. *Acta Hort*. (ISHS) 683: 399-406.

Rademacher W. (1995): Growth retardants: biochemical features and applications in horticulture. Acta Hort. (ISHS) 394: 57-74.

Tripathi, A. N., Tripathi S. N., Shukla R. K., Pandey, G. (2003): Effect of GA, NAA and CCC on growth and flowering of French marigold (*Tagetes patula*). *Journal of Applied Horticulture* 5(2): 112-113.

Welander N. T. (1984): Effect of GA_3, CCC defoliation and quantum flux density on growth and flowering in *Pelargonium × hortorum. Scientia Horticulturae*, 23 (4): 371-377.

THE IMPACT OF SUBSTRATE AND FERTILIZATION ON GROWTH AND DEVELOPMENT OF *Viola odorata* L.*

Renata Erhatić[1*], Vesna Židovec[2], Mirjana Herak Ćustić[2], Zdenko Lončarić[3], Marija Vukobratović[1], Štefica Ščetarić[1]

Original scientific paper

Summary

In order to achieve more successful breeding of the sweet violet, special attention should be given to the substrate and fertilization, since nitrogen affects the foliage mass, and phosphorus affect the better flowering. Better results are achieved when Viola species are grown in peat-based substrates than in soil-based. With the aim to obtain better information about selecting the most favourable substrate and the optimal dose of fertilizer, an investigation was conducted with five different types of commercial substrates, garden soil and soil from natural habitats and the foliar nutrition in four fertilization treatments (control, 0.1% NPK-Mg, 0.2% NPK-Mg and 0.2% NPK-Mg + 4% $MgSO_4$). Chemical analysis of the soil showed that the soil with less organic matter, higher pH reaction and richer in potassium compared to a commercial substrate. The results of the measurements show that the plants grown in soil were larger in diameter and height. Fertilization increased plant diameter by 6%, while plant height was not affected. Selection of the most appropriate substrate and optimal fertilization indirectly reduce the destruction of this nutritionally valuable and ornamental plant species in its natural habitats.

Keywords: *growing media, morphometric properties, colour of leaves*

INTRODUCTION

Sweet violet is a well known medicinal, edible and ornamental plant which blooms with flowers of attractive scent early in the spring, in the conditions of continental climate (Erhatić, Vukobratović *et al.* 2010). It grows wild in nature, in places exposed to sun, alongside hedges, river banks, on the edges of deciduous forests and in forest glades (Willfort 2002, Černicki 2006). It is wide–spread all across Croatia, along with the mentioned species, Domac (2002) lists further 19 species of the same genus. Soils from natural habitats contain a significant amount of organic matter (6-11%). Organic matter has a positive effect on plant growth because it

*Rad prezentiran na 25. Međunarodnom naučno-stručnom kongresu poljoprivrede i prehrambene industrije/ Paper presented at the 25th International Scientific-Expert Congress on Agriculture and Food Industry, 25-27 September 2014, Izmir, Turkey
[1] Križevci College of Agriculture, M. Demerca 1, HR-48260 Križevci, Croatia
[2] University of Zagreb Faculty of Agriculture, Svetošimunska cesta 25, 10000 Zagreb, Croatia
[3] Faculty of Agriculture in Osijek, University J. J. Strossmayer, Kralja Petra Svačića 1d, Osijek, Croatia
*rerhatic@vguk.hr

contributes to the improvement of the physical, chemical and biological soil properties, mineralization of nutrients and therefore, on the plant growth itself (Makineci *et al*. 2007). Violets are usually found on soils with less phosphorus and larger potassium content (Makineci *et al*. 2007, Bagci *et al*. 2008). When growing plants in substrates, peat and compost based substrates gave the best results: the greatest height of plants, the greatest mass and leaf surface, the longest root. Substrates based on soil, however, gave the weakest results Erhatić, Vukobratović *et al*. (2010).

The aim of this study was to investigate the influence of the substrate, fertilization and their interaction on some morphometric characteristics of sweet violet: plant height, diameter and colour of leaves and flowers.

MATERIALS AND METHODS

In June 2009 plants have been propagated by dividing and 320 plants per soil or substrate type were planted in two soil types (forest and garden soil Križevci area) and five different substrates produced by mixing different basic raw materials (Table 1). During the research, violets were fertilized in four levels of fertilization: variant without adding fertilizer (C), “KRISTALON“ - 0,1% NPK-Mg lower level (NPK-L), „KRISTALON“ - 0.2% NPK-Mg higher level (NPK-H), „KRISTALON“ - 0.2% NPK-Mg higher level with the addition of 4% $MgSO_4$ NPK-Mg). A two factorial experiment was set in a randomized block design with four replications, and each combination was represented with 20 plants. During winter of 2010 and spring of 2011, the following morphometric properties of sweet violet plants were measured: plant height (cm), diameter of plants (cm) and colour of leaves and flowers. Leaf and flower colour was determined by the RHS colour chart. Fertilizer was applied only twice (June 9th and June 25th, 2011). Due to low temperatures and bad weather conditions it was not possible to fertilize the plants earlier. The effects of substrate, fertilization and their interactions were tested with a two-way variance analysis.

The results of analysis of fertilizers and compost were statistically analyzed by SAS PC applications for Windows (SAS Institute Inc., Cary, NC, USA), Excel and StatSoft Statistica for determining the analysis of the variance (ANOVA).

RESULTS AND DISCUSSION

Substrate analysis

The chemical properties of substrates and soils are shown in Table 1. The reaction of the substrates ranged from pH 5.5 to 5.8, and the pH of soils from 7.1 to 7.7. According to the measured pH reaction, the substrates are defined as mildly acidic, while the soils are neutral. Although this reaction is suitable for the cultivation of most plant species, it seems that the sweet violet prefers a higher pH so the plants grown in forest soil had the greatest diameter and maximum height. The measured electrical conductivity (EC) is in the range from 0.35 to 0.90 dS m^{-1}. Low EC can mean a low fertility, because of the low nutrient content, but on the other hand, a high salt concentration may indicate potential phytotoxicity. Since the desirable value of EC for media that are intended for breeding of plants ranges from 0.5 to 1.8 dS m^{-1} (Kuo, 2004), we conclude that the substrates in the study were suitable for growing violets. The substrates and soils were rich in organic matter (Table 1). The content of basic macroelements in substrates varied in a very wide range (N 100-280 mg l^{-1}; P_2O_5 130-240 mg l^{-1} and K_2O 180-360 mg l^{-1}), and the soil was well supplied with nitrogen, poorly supplied with phosphorus and medium to richly supplied with potassium.

Plant diameter and height

Measurement results (Table 2) show a statistically important difference in plant diameter, depending on the type of substrate and the date of measuring ($P<0.0001$). The average maximum diameter of the plants independent of the measurement date was found on the forest soil (11.40 cm). Statistically significant difference in plant diameter was registered amongst substrates and fertilization treatments (Table 3). Plants grown on forest soil and garden soil had the biggest diameter, whilst those grown on commercial substrates had smaller diameters. Depending on fertilization, the best results were achieved using NPK-Mg treatment.

There is also statistically important difference between plant heights depending on substrates and measuring dates (Table 4). The highest plants were measured on forest soil (5.69 cm), and the shortest plants on garden soil (3.89 cm). After the fertilization, the highest plants were developed on forest soil (8.80 cm), and they showed a slight difference compared to the plants grown on the substrate S_5 (8.04 cm). Significantly lower plants were developed on the substrates S_3 and S_4, and they were comparable to the plants grown on the garden soil, and substrates S_1 and S_2. Different fertilization had no significant impact on plant height (Table 5). Dubsky and Sramek (2007) and Vukobratović (2008) state that better results of growth parameter (height and mass of plant) were achieved on peat-based substrates and compost rather than soil-based ones. The impact of foliar fertilization on flower height was researched by Ćustić and Poljak (1994) and it shows better results in the application of fertilizer.

Colour of leaves and flowers

The most common leaf colours before and after fertilization are 137A and 138A green and the most common flower colour was 86 A purple, which coincides with the research of Židovec (2009).

Table 1. Chemical properties substrates and soils

Substrates	pH_{H2O} (1:10)	EC dSm^{-1} (1:5)	Organic matter (%)	N mg l^{-1}	P_2O_5 mg l^{-1}	K_2O mg l^{-1}
Substrate 1	5.8	0.35	80	150	170	190
Substrate 2	5.8	0.60	90	280	200	360
Substrate 3	5.6	0.90	85	100	130	180

Substrate 4	5.5	0.45	80	210	150	270
Substrate 5	5.8	0.50	90	210	240	280
Soils	pH_{H2O}	pH_{KCl}	Humus (%)	N	AL-P_2O_5	AL-K_2O
				mg kg^{-1}		
Forest soil (F_s)	7.7	6.8	4.93	3.8	3.82	15.54
Garden soil (G_s)	7.1	6.5	4.57	3.5	3.47	68.95

Table 2. The diameter of the plants during the growing season of sweet violets per substrates before fertilization (cm).

Substrate/date	F_S	Gs	S_1	S_2	S_3	S_4	S_5	Average
14/10/2010	14.90	14.70	13.80	12.00	12.50	11.40	14.20	13.36 A
9/11/2010	12.90	11.00	12.00	9.80	12.30	11.00	13.20	11.73 B
15/12/2010	10.70	10.30	8.30	7.50	8.50	9.20	13.60	9.73 C
19/01/2011	10.10	9.30	7.40	6.80	8.40	7.70	11.20	8.69DE
21/02/2011	10.00	9.20	6.70	6.70	7.80	6.80	8.80	8.00 FG
17/03/2011	10.20	9.10	6.60	6.40	7.70	6.60	7.70	7.77 G
15/04/2011	10.50	9.00	7.40	7.30	7.50	6.80	9.80	8.33 FE
12/05/2011	11.50	9.30	9.60	8.50	8.40	8.70	7.60	9.06 D
30/05/2011	11.70	10.40	11.00	8.40	10.80	8.80	9.90	10.13 C
Average	11.40 A	10.24 B	9.20 C	8.15 D	9.31 C	8.55 D	10.67 B	9.64

Means separation at 5% level; capital letters between date mean values, small letters between substrate types values.

Table 3. Diameter of sweet violet plants after fertilization performed on substrates (cm)

Substrate/ fertilizer	F_S	G_S	S_1	S_2	S_3	S_4	S_5	Average
Control	11.90 ns	13.00 ns	12.70 ns	10.90 ns	12.20 ns	10.70 ns	12.60 ns	11.72 B
NPK - L	14.40 ns	13.10 ns	13.00 ns	10.20 ns	11.60 ns	10.60 ns	13.70 ns	11.88 AB
NPK - H	12.20 ns	13.80 ns	11.00 ns	12.50 ns	12.00 ns	11.30 ns	11.10 ns	11.86 AB
NPK-Mg	14.50 ns	12.30 ns	12.70 ns	11.10 ns	12.90 ns	11.10 ns	11.80 ns	12.45 A
Average	13.21 A	13.02 A	12.34 AB	11.18 B	12.18 AB	10.90 B	12.26 AB	11.98

Means separation at 5% level; capital letters between fertilizer mean values, small letters between substrate types values.

Table 4. Height of plants during the growing season of sweet violets per substrates before fertilization (cm)

Substrate/date	F_S	G_S	S_1	S_2	S_3	S_4	S_5	Average
14/10/2010	6.50	5.90	5.90	6.10	5.20	6.30	5.00	5.84 A
9/11/2010	4.90	4.50	4.50	5.40	3.60	5.80	5.30	4.85 C

15/12/2010	5.20	3.80	3.30	3.00	2.80	3.30	4.10	3.63 E
19/01/2011	5.20	3.30	2.80	2.60	2.90	3.20	3.20	3.31 F
21/02/2011	5.20	3.10	2.70	2.90	2.50	3.10	3.20	3.24 F
17/03/2011	5.40	3.10	2.70	3.30	4.40	3.30	3.00	3.60 E
15/04/2011	5.70	3.20	4.00	4.20	4.30	3.90	5.20	4.35 D
12/05/2011	6.30	3.70	5.50	5.40	5.50	4.90	5.50	5.27 B
30/05/2011	6.70	4.40	5.90	5.90	6.00	4.90	6.10	5.69 A
Average	5.69 A	3.89 D	4.15 C	4.29 BC	4.13 CD	4.28 BC	4.51 B	4.42

Means separation at 5% level; capital letters between date mean values, small letters between substrate types values.

Table 5. Plant height of sweet violets upon completion of fertilization per substrates (cm)

Substrate/ fertilizer	F_S	G_S	S_1	S_2	S_3	S_4	S_5	Average
Control	8.20 ns	7.20 AB	7.00 ns	7.30 ns	6.00 ns	6.90 ns	6.70 ns	7.38 ns
NPK - L	9.60 ns	6.50 B	7.50 ns	8.80 ns	6.70 ns	6.90 ns	8.70 ns	7.38 ns
NPK - H	8.30 ns	7.90 A	8.50 ns	6.90 ns	7.10 ns	7.00 ns	9.10 ns	7.49 ns
NPK-Mg	9.50 ns	6.80 B	7.70 ns	7.40 ns	7.40 ns	6.60 ns	7.70 ns	7.37 ns
Average	8.80 A	7.08 BC	7.66 BC	7.60 BC	6.78 C	6.84 C	8.04 AB	7.41

Means separation at 5% level; capital letters between fertilizer mean values, small letters between substrate types values.

CONCLUSIONS

The observation of the impact of the substrate and fertilisation on the growth of the sweet violet has only partially confirmed the hypothesis of the vegetative growth of the sweet violet being greater on the substrate with more organic compounds. Only the height and the diameter of sweet violet plants were greater on the substrate with less organic compound (garden soil and forest soil), while the leaf number was greater on the substrate with more organic compound.

The same situation repeated with the pH of the tested substrates. The hypothesis of the vegetative growth of sweet violet plants being greater on the substrate with a lower pH value was also ony partially confirmed. Only the height and the diameter of sweet violet plants were greater on the substrate with a greater pH reaction (garden soil and forest soil), while the number of the leaves was greater on the substrate with a lower pH reaction. Based on the results of the conducted research, it may be concluded that the most favorable medium for the cultivation of sweet violet is the forest soil, along with the fertilizer NK – higher level and NPK – higher level with the addition of Mg.

The observation of the impact of the substrate and fertilisation on the growth of the sweet violet has only partially confirmed the hypothesis of the vegetative growth of the sweet violet being greater on the substrate with more organic compounds. Only the height and the diameter of sweet violet plants were greater on the substrate with less

organic compound (garden soil and forest soil), while the leaf number was greater on the substrate with more organic compound.
The same situation repeated with the pH of the tested substrates. The hypothesis of the vegetative growth of sweet violet plants being greater on the substrate with a lower pH value was also ony partially confirmed. Only the height and the diameter of sweet violet plants were greater on the substrate with a greater pH reaction (garden soil and forest soil), while the number of the leaves was greater on the substrate with a lower pH reaction. Based on the results of the conducted research, it may be concluded that the most favorable medium for the cultivation of sweet violet is the forest soil, along with the fertilizer NK – higher level and NPK – higher level with the addition of Mg.

REFERENCES

Bağci, Y., Dĭnc, M. and Őztűrk, M. 2008. Morphological, Anatomical and Ecological Study on Turkish Endemic Viola yildirimlii M. Dinc & Y. Bağc.International Journal of Natural and Engineering Sciences, 2 (3):01-05.

Černicki, L. 2006. Samoniklo cvijeće grada Zagreba. Školska knjiga, Zagreb, 150.

Ćustić, M. and Poljak, M. 1994. Effect of the liquid mineral fertilizer Fertina on *Tagetes patula nana* L. Agriculturae conspectus scientificus, 59 (1): 105-112.

Domac, R. 2002. Mala flora Hrvatske i susjednih područja. Školska knjiga, Zagreb.

Dubský, M. and Sråmek F. 2009. Substrates with mineral components for growing woody plants. Acta Hort. (ISHS) 819: 243-248.

Erhatić, R., Kajin, K., Mesić, Ž., Markovina, J., Žutić, I. and Židovec, V. 2010. Povijest i mogućnosti primjene mirisave ljubičice. Agronomski glasnik, Vol 72, No1: 47-58.

Erhatić, R., Vukobratović, M., Peremin Volf, T. and Židovec, V. 2010. Morphological and Chemical Properties of Selected Sweet Violet Populations. Journal of Central European Agriculture, Vol. 11, No 1: 55-64.

Kuo, S., Ortiz-Escobar, M. E., Hue, N. V. and Hummel, R. L. 2004. Composting and compost utilization for agronomic and container crops. Recent Res Devel Envirnomental Biology, 1: 451-513.

Makineci, E., Demir, M., Comez, A. and Yilmaz, E. 2007. Chemical characteristics of the surface soil, herbaceous cover and organic layer of a compacted skid road in a fir (*Abies bornmulleriana* Mattf.) forest. Transportation Research Part D 12: 453-459.

Vukobratović, M. 2008. Proizvodnja i ocjena kvalitete kompostiranih stajskih gnojiva. Doktorska disertacija, Poljoprivredni fakultet u Osijeku

Willfort, R. 2002. Ljekovito bilje i njegova upotreba. Erudit d.o.o., Zagreb.

Židovec, V. 2009. Proizvodnja mirisave ljubičice (*Viola odorata* L.). Završno izvješće IV. 2006 – IV. 2008, Agronomski fakultet Sveučilišta u Zagrebu.

BIOFERTILIZATION AND ITS EFFECT ON SOIL MICROBIAL DIVERSITY AND SOME MORPHOLOGICAL PROPERTIES OF SOYBEAN*

BIOFERTILIZACIJA I NJEN UTICAJ NA MIKROBNI DIVERZITET ZEMLJIŠTA I NEKE MORFOLOŠKE OSOBINE SOJE

Saud Hamidović[1], Elvira Karić[1], Drena Gadžo[1], Blažo Lalević[2], Hadi Waisi[3], Josip Čolo[1], Teofil Gavrić[1]

Original scientific paper

Summary

In contemporary agriculture production, using of fertilizers is necessairy for obtaining of high yield of soybean. Except of organic and mineral fertilizers, microbiological fertilizers can also be applied in soybean production. The aim of this paper was to determined the influence of two biofertilizers (Ekstrasol and Megaflu) on microbial characteristics of soil and morphological properties of soybean. Sampling for microbiological and chemical analyses was achieved at Butmir (Sarajevo canton, Bosnia and Herzegovina) in spring 2016 in the phases of sowing, flowering and physiological maturity of soybean. At the end of experiment, plant and root lenght and weight, as well number of nodules, legumes and seeds per legume, seed weight were determined. Lowest microbial number was detected in the time of sowing seeds, and highest in physiological maturity. In most of samples, in control and Megaflu treatment, higher microbial activity compared with Ekstrasol was noticed. In treatment with Megaflu, plant and root weight, number of nodules, legume number per plant, seed number and weight per plant were higher compared to control and Ekstrasol treatment. In other morphological parameters, differences between the treatments were not detected. This study can be useful for improvement of soybean production and further application of microbiological fertilizers.

Key words: *biofertilization, microbial diversity, soybean*

Rezime

U savremenoj poljoprivrednoj proizvodnji, neophodna je upotreba đubriva u cilju dobijanja visokih prinosa soje. Osim organskih i mineralnih đubriva, mikrobiološka đubriva se takođe mogu koristiti u proizvodnji soje. Cilj ovog rada bio je

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] University of Sarajevo, Faculty of Agriculture and Food Sciences, Bosnia and Herzegovina
[2] University of Belgrade, Faculty of agriculture, Belgrade - Zemun, Serbia
[3] Institute for Water Management "Jaroslav Cerni", Belgrade, Serbia

determinacija uticaja dva biofertilizatora (Ekstrasol i Megaflu) na mikrobiološke osobine zemljišta i morfološke osobine soje. Uzorkovanje za mikrobiološke i hemijske analize obavljeno je na lokaciji Butmir (Sarajevski kanton, Bosna i Hercegovina) u proljeće 2016. godine u fazama sjetve, cvjetanja i fiziološke zrelosti soje. Na kraju ogleda, mjerena je dužina biljke i dužina korijena, kao i broj kvržica, mahuna i sjemena po mahuni i težina sjemena. Najmanja mikrobiološka aktivnost zabilježena je u fazi sjetve, a najveća u fazi zrelosti. U najvećem broju uzoraka, u kontroli i tretmanu sa biofertilizatorom Megaflu bila je veća mikrobiološka aktivnost u poređenju sa tretmanom Ekstrasol-om. U tretmanu sa Megaflu-om dužina biljaka i korijena, broj kvržica, broj mahuna po biljci, broj sjemena i njihova težina po biljci bili su veći u poređenju sa tretmanom Ekstrasol-om. U ostalim parametrima rasta, razlike nisu ustanovljene. Ova istraživanja mogu poslužiti za unapređenje proizvodnje soje i dalju aplikaciju mikrobioloških đubriva.

Ključne riječi: *biofertilizacija, mikrobna raznolikost, soja.*

INTRODUCTION

The soybean plant (*Glycine max* L. Merr) is economically very important crop, which serves as a protein source for humans and livestocks (Friedman and Brandon, 2001). Soybean seeds contain about 36 to 38% of proteins and 19% of oil (Macak and Candrakova, 2013), but these percents strongly depend on various factors during cultivation (Krishnan, 2001). Except of proteins and oil, seeds also contain isoflavones (Horn-Ross *et al*., 2000), phytic acid and oligosaccharides (Liener, 1994), having beneficial impact on human nutrition and health, such as lowering of cholesterol, prevention of cancer and diabetes etc. (Friedman and Brandon, 2001).

Sustainable contemporary agriculture production is characterized by the presence of diverse crops in agroecosystems. Because of it, soil fertility and optimal nutrient content are necessairy for successfull crop production (Macak and Candrakova, 2013). However, in some cases, unfavourable environmental conditions may limit the productivity of soybean plants, as well as other legumes. Legumes are often grown after corn harvest and because of relatively short vegetation season, potential problems during the cultivation may be linked with drough and soil degradation (Graham and Vance, 2003). Thus, for the solving of these practical problems, incorporation of fertilizers may help in improvement of crop yield (Basso and Ritchie, 2005).

Soybean may use nitrogen released during mineralization, from residua of soil living organisms, fertilizers or nitrogen from atmosphere, which can be transformed to available form. This process occurs due to symbiose between soybean plant and bacteria *Bradyrhizobium japonicum*. Through the nitrogen fixation, soybean obtains 65-85% of its needs (Rao and Reddy, 2010). Also, in this plant-microbial association, soybean can fix up to 200 kg N/ha per year, which is important for reduction of the nitrogen fertilizers application (Javaid and Mahmood, 2010). On the other side, many bacterial species, such as rhizobacteria, possess plant growth

promoting (PGP) mechanisms. Products of metabolytic activity of rhizobacteria (auxins, cytokinins and gibberellins) are responsible for direct promotion of plant growth (Suresh *et al*., 2010).
Microbiological fertilizers contain one microorganism type or a consortium of microorganisms. Application of this type of fertilizers is environmental-friendly and low-cost method compared to conventional fertilizers (Milić *et al*., 2003); this is promising technology in improvement of crop quality and protection from different undesirable factors (Yang *et al*., 2009).
The aim of this paper was to detemined the influence of microbiological fertilizers on microbial diversity in soil and parameters of morphological properties of soybean.

MATERIAL AND METHODS

The experiment was performed in May 2016 at Butmir location (Sarajevo canton, Bosnia and Herzegovina) by inoculation of soybean seeds (Balkan, first maturity group). For inoculation, two microbiological fertilizers were used: Ekstrasol (Krasnodar, Russia), containing pure cultures of Bacillus subtilis, and Megaflu (Biomarket, Turkey), containing pure cultures of bacteria *Bacillus megaterium*, *Pantoea agglomerans* and *Pseudomonas fluorescens*. Inoculation was performed according to producer guide. In control were used uninoculated seeds.
Soil samplings were achieved after sowing, in the time of flowering (August 2016) and physiological maturity (October 2016). Sampling was performed from depth 0-30 cm. Chemical analyse of soil used for experiment was performed using standard methodology. pH value in water and 1M KCl was determined by ISO standard 10390, humus content by dichromate method (Walkley, 1935), available content of K and P by AL method (Egner *et al*., 1960), and carbonate content using the Scheibler calcimeter.
After preparation of composite sample from each treatment, microbiological analyses were performed using standard methodology.
Total number of bacteria (TNB) was determined using 0,1xTSA, ammonification bacteria (AMB) using nutrient agar, nitrogen fixation bacteria (NFB) and *Azotobacter* sp. (AZ) on Fyodorov agar, fungi (F) on rose bengal streptomycin agar (Peper *et al*., 1995), and actinomycetes (AM) on starch-ammonia agar. After incubation (for fungi at 25°C for 5 days, for *Azotobacter* sp. at 28°C for 2 days, and for other bacteria at 28°C for 6 days), microbial activity of samples was expressed in colony forming units (CFU) per gram of absolutely dry samples.
Plants sampling was performed in August and October 2016. In all samplings, eight plants were selected for research of morphological parameters. In first sampling, whole plant lenght and weight, root lenght and weight, total number of nodules and number of active nodules were determined. In second sampling, legume number, number of seeds in legume and seed weight per plant were determined.

RESULTS AND DISCUSSION

Results of chemical analyse weakly acidic pH / KCl value of soil, neutral pH/H_2O value of soil, low humus content. The soil is well provided with available phosphorus and medium low with available potassium (Tab. 1).

Tab. 1. Chemical analyse of soil under soybean

parameters	pH in H_2O	pH in KCl	humus (%)	P_2O_5 (mg/100g)	K_2O (mg/100g)	$CaCO_3$ (%)
values	6.81	5.66	2.34	16.53	13.6	0.0

Plant-microbial interactions are very complex and sometimes have significant consequences for the plant growth. Some bacteria living in the rhizosphere are able to stimulate nodule formation and biological nitrogen fixation of legumes (Kravchenko *et al.*, 2013), which have also stimulatory effect on the presence of other groups of microorganisms. Activity of microbial populations in the soil can be an important parameter of soil fertility, because they contribute to the humus synthesis and mineralization (Balešević-Tubić *et al.*, 2011).

Our results showed that number of microorganisms depends on time of sampling and fertilizers application (tab. 2, 3 and 4).

Tab. 2. Microbial diversity in soil under soybean after soybean sowing

Treatments	TNB	AMB	NFB	F	AM	AZ
	($x10^5$ CFU/g)			($x10^3$ CFU/g)		($x10^2$ CFU/g)
Ekstrasol	42.5	12.5	6.5	21.7	33.9	28.4
Megaflu	25.0	24.2	14.7	24.3	31.3	47.8
Control	52.1	13.0	15.2	23.4	52.3	32.1

Most abundant microbial population in our research are bacteria. In all samples, abundance of bacteria was comparatively higher than fungi and actinomycetes. Similar results were obtained by Zhao *et al*. (2013), who suggest that bacteria represent no lower than 95% of all microorganisms in soil ecosystems.

As can be seen form table 2, total number of bacteria is most abundant microbial population in soil. These bacteria play important role in soil fertility and mineralization of organic matter (Jarak and Čolo, 2007). In control, activity of these bacteria was higher compared to fertilizer treatments. Similar results were obtained for NFB and AM. On the other side, in treatment with Megaflu, highest number of AMB, F, and AZ was detected.

Tab. 3. Microbial diversity in soil under soybean during soybean flowering

Treatments	TNB	AMB	NFB	F	AM	AZ
	($x10^5$ CFU/g)			($x10^3$ CFU/g)		($x10^2$ CFU/g)
Ekstrasol	61.9	25.5	17.0	38.7	57.6	20.1
Megaflu	51.5	15.0	10.7	21.5	66.2	33.5
Control	94.5	20.0	16.1	30.9	55.0	42.4

During the flowering, in almost all samples, increase of microbial activity was observed compared to first sampling. In control treatment, number of TNB and AZ was highest compared to fertilization treatments. In treatment with Ekstrasol, highest activity of AMB, NFB and F was noticed, while abundance of AM was highest in treatment with Megaflu (Tab. 3).

In most of samples, microbial activity was higher compare to first and second sampling (Tab. 4). Highest microbial activity in control was obtained at TNB and F. In Ekstrasol treatment, number of AMB was higher compared to other treatments, while in Megaflu treatment, number of other microbial group was highest compared to control and Ekstrasol treatment.

Tab. 4. Microbial diversity in soil during physiological maturity of soybean

Treatments	TNB	AMB	NFB	F	AM	AZ
	($x10^5$ CFU/g)			($x10^3$ CFU/g)		($x10^2$ CFU/g)
Ekstrasol	66.0	34.3	13.5	30.0	83.2	46.1
Megaflu	31.0	23.4	15.9	23.1	102.9	67.3
Control	84.0	21.7	15.4	37.7	62.6	38.3

From these data can be seen that application of microbiological fertilizers had no effect on number of microorganisms. This statement can be linked with absence of other nutrient sources, such as mineral or organic fertilizers. Crecchio *et al*. (2004) and Marschner *et al*. (2001) have reported that at some experiments without appropriate use of fertilizers, changes of bacterial community were not observed. On the other side, Balešević-Tubić *et al*. (2011) were noticed the increase of microbial abundance in soil fertilized by mineral and microbiological fertilizers. Also, increase of microbial diversity and structure of microbial community was recorded using organic and microbiological fertilization in previous studies (Franzluebbers *et al*., 2004; Javorekova *et al*., 2015).

Tab. 5. Plant and root characteristics of soybean in experiment

Treatments	Plant lenght (cm)	Plant weight (g)	Root lenght (cm)	Root weight (g)
Ekstrasol	118.0	133.8	23.9	10.2
Megaflu	118.1	159.4	22.9	18.7
Control	125.1	167.8	24.8	14.8

As can be seen from Table 5, in most of treatments, highest values of examinated parameters of soybean growth was observed in control variant. Shahid *et al*. (2009) also confirmed similar results during inoculation of soybean seeds with *B. japonicum*, where average plant lenght in inoculated treatment was 49.6 cm, while in control 47.05 cm, without statistically significance of obtained results. This statement is in agreement with our results. Using *Bacillus subtilis*, significant differences in root lenght between inoculated and inoculated plants were not noticed (Araujo *et al*., 2005).

Since 1970s is known that using *Rhizobium* bacteria lead to the nodules formation on plant roots (Bhuvaneswari *et al*., 1981). The same authors confirmed that number of nodules increased with increase of soil depth. In all samples, number of nodules and legumes, as well as seed number per plant and seed weight was higher in treatment with Megaflu compared to Ekstrasol and control.

Tab. 6. Nodules, legume and seed characteristics in experiment

Treatments	Average number of nodules		Legume number per plant	Seed number per legume	Seed weight per plant (g)
	total	active			
Ekstrasol	4.9	4.4	79.3	2.3	34.1
Megaflu	6.6	5.4	106.5	2.5	51.3
Control	1.9	1.7	78.8	2.4	36.0

Positive effects of biofertilizers inoculation on some morphological characteristics of soybean was obtained in previous research (Abbasi *et al*., 2008). In Croatia, inoculation of soybean seeds with B. japonicum showed stimulatory effect on average legumes number - 33.07 in inoculated treatment and 20.67 in control (Pušić *et al*., 2008). Bhuiyan *et al*. (2008) confirmed that seeds number per plant was higher in inoculated tretment compared to control.

CONCLUSION

This study demonstrated the influence of biofertilizers on soil microbial activity and soybean characteristics. Microbial activity was lower in first sampling (sowing), higher during the flowering phase and highest in the time of physiological maturity. However, differences in microbial diversity per treatments were not observed. Highest number of nodules, root weight, number of legumes per plant and seeds weight per plant was noticed in treatment with Megaflu. Further research will be done in order to determinate the influence of used biofertilizers in combination with mineral/organic fertilizers on microbial diversity of soil and soybean characteristics.

ACKNOWLEDGEMENTS

This research is partially supported by Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant No. TR 31080.

BIBLIOGRAPHY

Abbasi, K. M., Majeed, A., Sadig, A., Khan, R. S. (2008): Application of *Bradyrhizobium japonicum* and phosphorus fertilization improved growth, yield and nodulation of soybean in the sub-humid Hilly Region of Azad, Jamma and Kashmir, Pakistan. Plant Production Science, 11(3): 368-376.

Araujo, F. F., Henning, A. A., Hungaria, M. (2005): Phytohormones and antibiotics produced by Bacillus subtilis and their effects on seed pathogenic fungi and on Soybean root development; World Journal of Microbiology and Biotechnology, 21: 1639-1645.

Balešević-Tubić, S., Đukić, V., Marinković, J., Dozet, G., Petrović, K., Tatić, M. (2011): Importance of microbiological fertilizer used in soybean production: Agronomical and biological aspects. African Journal of Microbiology Research, 5(27): 4909-4916.

Basso, B., Ritchie, J. T. (2005): Impact of compost, manure and inorganic fertilizer on nitrate leaching and yield for a 6-year maize-alfalfa rotation in Michigan. Agriculture, Ecosystems and Environment, 108(4): 309-341.

Bhuiyan, M. A. H., Mian, M. H., Islam M. S. (2008): Studies on effects of *Bradyrhizobium* inoculation on yield and yield attributes of mung bean. Bangladesh Journal of Agricultural Research, 33: 449-457.

Buhvaneswari, T. V., Bhagwat, A. A., Bauer, W. D. (1981): Transient susceptibility of root cells in four common legumes to nodulation by rhizobia; Charles F. Kettering Research Laboratory, Yellow Springs, Ohio.

Crecchio, C., Curci, M., Pizzigallo, M. D. R., Ricciuti, P., Ruggiero, P. (2004): Effects of municipal solid waste compost amendments on soil enzyme activities and bacterial genetic diversity. Soil Biology and Biochemistry, 36: 1595-1605.

Egner, H., Riehm, H., Domingo, W. R. (1960): Untersuchungen uber die chemische Boden analyse als Grundlage fur die Beurteilung des Nahrstoffzustandes der Boden, II: Chemische Extractions metoden zu Phosphor und Kalium bestimmung. Kungliga lantbrukshugskolans annaler, 26: 199-215.

Franzluebbers, A. J., Wilkinson, S. R., Stuedemann, J. A. (2004): Bermudagrass management in the Southern Piedmont, USA: IX. Trace elements in soil with broiler litter application. Journal of Environmental Quality, 33: 778-784.

Friedman, M., Brandon, D. L. (2001): Nutritional and health benefits of soy proteins. Journal of Agricultural and Food Chemistry, 49(3): 1069-1086.

Graham, P. H., Vance, C. P. (2003): Legumes: importance and constraints to greater use. Plant Physiology, 131: 872-877.

Horn-Ross, P. L., Barnes, S. Lee, M., Coward, L., Mandel, J. E., Koo, J., John, E. M., Smith, M. (2000): Assessing phytoestrogen exposure in epidemiologic studies: development of a database (United States). Cancer Causes Control, 11: 289-298.

Jarak, M., Čolo, J. (2007): Mikrobiologija zemljišta. Poljoprivredni fakultet, Novi Sad.

Javaid, A., Mahmood, N. (2010): Growth, nodulation and yield response of soybean to biofertilizers and organic manures. Pakistan Journal of Botany, 42(2): 863-871.

Javorekova, S., Makova, J., Medo, J., Kovacsova, S., Charousova, I., Horak, J. (2015): Effect of bio-fertilizers application on microbial diversity and physiological profiling of microorganisms in arable soil. Eurasian Journal of Soil Science, 4: 54-61.

Kravchenko, I., Kizilova, A., Titova, L., Iutinskaya, G. (2013): Effect of microbial fertilizers on rhizospheric bacterial diversity and yield response of soybean plants. Agriculture Science Developments, 2(12): 120-125.

Krishnan, H. B. (2001): Biochemistry and molecular biology of soybean seed storage proteins. Journal of New Seeds, 2(3): 1-25.

Liener, I. E. (1994): Implications of antinutritional components in soybean foods. Critical Reviews of Food Science and Nutrition, 34: 31-67.

Macak, M., Candrakova, E. (2013): The effect of fertilization on yield components and quality parameters of soybeans [(Glycine max (L.) Merr.] seeds. Journal of Central European Agriculture, 14(3): 379-389.

Marschner, P., Yang, C. H., Lieberei, R. Crowley, D. E. (2001): Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biology and Biochemistry, 33: 1437-1445.

Milić, V., Hrustić, M., Vasić, M., Starčević, Lj., Marinković, J. (2003): Use of microbiological fertilizers in bean, soybean and maize production. Field Vegetables Crop Research, 38: 259-270.

Peper, I. L., Gerba, C. P., Brendencke, J. W. (1995): Environmental microbiology. Academic Press, San Diego, 11-33.

Pušić, D., Kristek, S., Kristek, A., Antunović, M. (2008): Uticaj kvržičnih bakterija i mikoriznih gljiva na elemente prinosa soje, Zbornik radova 43. Hrvatskog i 3. Međunarodnog simpozija agronoma, Sveučilište u Zagrebu, Agronomski fakultet Zagreb, Zagreb, pp. 619-623.

Rao, A. S., Reddy, K. S. (2010): Nutrient management in soybean. In: The soybean: botany, production and uses (Singh, G., Ed.). CAB International, pp. 1-30.

Shahid, M. Q., Saleem, M. F., Khan, Z. H., Anjum, A. S. (2009): Performance of Soybean (*Glycine max.* L.) under different phosphorus levels and inoculation; Pakistan Journal of Agricultural Sciences, 46(4): 237-241.

Suresh, A., Pallavi, P., Srinivas, P., Kumar, P. V., Chandra, J. S., Reddy, R. S. (2010): Plant growth promoting activities of fluorescent pseudomonads associated with some crop plants. African Journal of Microbiology Research, 4(14): 1491-1494.

Walkley, A. (1935): An examination of methods for determining organic carbon and nitrogen in 15 soils. Journal of Agricultural Sciences, 25: 598-609.

Yang, J., Kloepper, J. W., Ryu, C. M. (2009): Rhizosphere bacteria help plants tolerate abiotic stress. Trends in Plant Science, 14: 1-4.

Zhao, Z. Q., Shahrour, I., Bai, Z. K., Fan, W. X., Feng, L. R., Li, H. F. (2013): Soils development in opencast coal mine spoils reclaimed for 1-13 years in the WestNorthern Loess Plateau of China. European journal of soil biology, 55: 40-46.

INFLUENCE OF HERBICIDE WING P ON MICROBIAL POPULATION DENSITY IN SOIL UNDER CORN*

UTICAJ HERBICIDA WING P NA GUSTINU MIKROBNE POPULACIJE U ZEMLJIŠTU POD KUKURUZOM

Saud Hamidović[1], Alma Žutić[1], Mirha Đikić[1], Josip Jurković[1], Vera Raičević[2], Milan Pešić[3], Amer Sunulahpašić[4]

Original scientific paper

Summary

Contemporary agricultural production is often linked with presence of pests, pathogens and weeds. Because of importance of yield quantity and quality, herbicides was introduced in plant production. However, the increased use of herbicides in agriculture has negative consequences for living organisms. The aim of this paper was to determinate the impact of herbicide Wing P on microbial activity of soil under corn (Pioneer hybrid PR37NO1). The experiment was performed in Ilidža municipality (Sarajevo canton, Bosnia and Herzegovina) by sowing seeds in May 2016, followed by Wing P application in three concentrations (2; 4; and 8 l ha^{-1}). Control treatment was untreated soil.

Chemical and microbiological characterization of soil was performed 15; 30; and 130 days from herbicide treatment using standard methodology.

The results of chemical analyses weakly acidic pH / KCl value of soil, neutral pH/H_2O value of soil, low humus content. The soil is well provided with available phosphorus and medium low with available potassium.Herbicide Wing P had no inhibitory effect on the activity of microbial population, except of actinomycetes and *Azotobacter* sp. These results suggest the possible adaptation of microbial population to various herbicide concentrations, which can be useful for further research of herbicide characteristics in plant production.

Key words: *herbicide, microbial population, corn*

Rezime

Savremena poljoprivredna proizvodnja je često vezana za prisustvo štetočina, patogena i korova. Zbog važnosti kvaliteta i kvantiteta prinosa, u biljnu proizvodnju je

---

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina

[1] University of Sarajevo, Faculty of Agriculture and Food Sciences, Bosnia and Herzegovina
[2] University of Belgrade, Faculty of agriculture, Belgrade - Zemun, Serbia
[3] Institute of soil science, Belgrade, Serbia
[4] Ministry of agriculture, water management and forestry of Central Bosnia Canton, Travnik, Bosnia and Herzegovina

uvedeno korišćenje herbicida. Međutim, njihova česta primjena u poljoprivredi ima negativne efekte na žive organizme. Cilj ovog rada bio je da se utvrdi uticaj herbicida Wing P na mikrobiološku aktivnost zemljišta pod kukuruzom (Pioneer PR37NO1). Ogled je postavljen na Ilidži (Sarajevski kanton, Bosna i Hercegovina) sjetvom sjemena u maju 2016. godine, nakon čega je primijenjen herbicid u tri koncentracije (2, 4 i 8 l ha^{-1}). U kontroli herbicid nije primijenjen. Hemijske i mikrobiološke karakteristike zemljišta izvršene su 15, 30, i 130 dana nakon tretmana herbicidima korišćenjem standardne metodologije.
Rezultati hemijskih analiza pokazuju kiselu pH vrijednost, osrednji sadržaj humusa i odsustvo karbonata. Zemljište je dobro obezbijeđeno pristupačnim fosforom i kalijumom. Herbicid Wing P nije pokazao negativno djelovanje na mikrobne populacije, izuzev na aktinomicete i *Azotobacter* sp. Ovi rezultati ukazuju na potencijalnu adaptaciju mikrobnih populacija različitim koncentracijama herbicida i mogu se koristiti za dalja istraživanja pri primjeni herbicida u biljnoj proizvodnji.

Ključne riječi: *herbicid, mikrobna populacija, kukuruz*

INTRODUCTION

The increasing human population and food demand leads to intensive plant production, concern of public health and adequate use of natural resources (Chowdhury *et al*., 2008). Contemporary technologies utilizing different management practices are inextricably linked with increasing of yield quality and quantity (Roger *et al.*, 1994). Unfortunately, according to IAEA (2001), crop losses from pests, pathogens and weeds was 28% in Europe. Because of this important problem in agricultural production, pesticides were introduced to protect crops from different undesirable factors (Yang *et al*., 2007). Their application has been largely expanded in several few decades; treatments include soil application, crop leaves and seed spray etc. (Sethi and Gupta, 2013). However, the increased use of these chemicals in agriculture production causes the soil pollution (Sebiomo *et al*., 2011) and has a negative consequences for flora and fauna, as well as for some chemical properties of soil, leading to the infertility of soil (Sarnaik *et al*., 2006). Their use also can affect the activity of microbial populations in soil (Singh and Singh, 2005). In short term researches, pesticides can stimulate, inhibit or have no effect on microbial abundance in soil (Grossbard, 1976). For example, Piotrowska-Seget *et al*. (2008) observed the reduction of bacterial activity after application of fungicide Captan. Li *et al.* (2010) noticed the initially stimulatory impact of herbicide Alachlor on fungal activity, followed with inhibition of fungal growth. Thus, effects of pesticides on microbial abundance in soil primarily depends on pesticides characteristics (Jacobsen and Hjelmso, 2014). On the other side, pesticides after their application, may be transformed by physical, chemical or biological processes, where microorganisms play an important role. The products of pesticides degradation can be accumulated on soil and microorganisms can remove and degrade them to enable their recycling in ecosystem (Chowdhury *et al.*, 2008).

In order to satisfy human needs, sustaining of ecosystem quality and resources, use of herbicides has great importance (Emurotu and Anyanwu, 2016). Some microorganisms use herbicides as a sole source of energy and nutrients, while to other microorganisms herbicides may be toxic (Ekschmitt and Griffiths, 1998). It is evident that some microbial populations responsible for herbicide degradation may exist in polluted locations, while others can reduce the soil pollution caused by herbicide application (Sebiomo *et al.*, 2011).

The aim of this research was to examine the effect of herbicide Wing P on microbial activity of soil under corn.

MATERIAL AND METHODS

The experiment was performed in May 2016 at Butmir location (Ilidža municipality, Sarajevo canton, Bosnia and Herzegovina) by sowing a corn seeds (Pioneer hybrid PR37NO1). After seeds sowing, treatment of soil by herbicides Wing P (BASF) obtained from local distributor was performed in orded to control the broad-leaved weeds and annual grasses. The concentrations of this herbicides were applied in this research: 2; 4; and 8 l ha^{-1}. Control treatment was soil without herbicide application.

Soil sampling was performed 15; 30; and 130 days after sowing seeds, from the depth 0 to 30 cm. In second and third sampling, samples was taken from the nearness of plants at several points. After the preparation of composite sample from each treatment, chemical and microbiological analyses were performed.

Measurement of pH value in water and 1M KCl was performed by ISO standard 10390, humus content by dichromate method (Walkley, 1935), available content of K and P by AL method (Egner *et al.*, 1960), and carbonate content using the Scheibler calcimeter.

Total number of bacteria was determined using 0,1xTSA, ammonification bacteria using nutrient agar, nitrogen fixation bacteria and *Azotobacter* sp. on Fyodorov agar, fungi on rose bengal streptomycin agar (Peper *et al.*,1995), and actinomycetes on starch-ammonia agar. Incubation for fungi was performed at 25°C for 5 days, for *Azotobacter* sp. at 28°C for 2 days, and for other bacteria at 28°C for 6 days. After incubation, microbial activity of samples was expressed in colony forming units (CFU) per gram of absolutely dry samples.

RESULTS AND DISCUSSION

The results of chemical analyses weakly acidic pH / KCl value of soil, neutral pH/H_2O value of soil, low humus content. The soil is well provided with available phosphorus and medium low with available potassium (Tab. 1).

Table 1. Chemical characteristics of soil

soil	pH		Humus	P_2O_5	K_2O	$CaCO_3$
	in H_2O	in KCl	(%)	(mg/100g)		(%)
	6.81	5.66	2.34	16.53	13.60	0.00

The results of this research showed different microbial activity depends on herbicide concentration and time of sampling.
Total number of bacteria is an important indicator of soil fertility (Jarak and Čolo, 2007). In almost all samples, total number of bacteria was higher in herbicide treatments compared to control (Tab. 2). Number of bacteria was highest after 130 days from sowing seeds. Except of treatment with 4 l ha^{-1} of herbicide, decrease of bacterial number in 30 days compared to 15 days after sowing was noticed. In all treatments, highest bacterial activity was noticed at the end of experiment.
Ammonification bacteria are responsible for degradation of organic matters in soil and theirs transformation into available forms of nitrogen. According to our results, highest number of ammonification bacteria was obtained 15 days after sowing seeds in treatment with 2 l ha^{-1} of Wing P (295.13 x 10^4 CFU/g). In this treatment, number of ammonification bacteria decreased during experiment. In treatment with 4 l ha^{-1}, highest number of these bacteria was noticed 30 days after sowing, while in other two treatments at the end of experiment (Tab. 2). Duah-Yentumi and Johnson (1986) confirmed that simazine caused no detectable impact on the microbial number, while paraquat significantly reduced microbial biomass after several soil treatments. Our results suggested the variability of effects on microbial abundance in soil, which is in agreement with previous researches (Chowdhury *et al.*, 2008).

Tab. 2. Total number of bacteria and ammonification bacteria number in soil

Treatments	Total number of bacteria (x 10^4 CFU/g)			Ammonification bacteria (x 10^4 CFU/g)		
	sampling after sowing					
	15 d	30 d	130 d	15 d	30 d	130 d
Wing P 2 l ha^{-1}	405.38	274.00	1179.61	295.13	182.12	57.46
Wing P 4 l ha^{-1}	228.29	334.13	1116.22	160.59	272.31	120.82
Wing P 8 l ha^{-1}	336.80	243.19	1127.46	198.78	131.45	200.67
control	266.49	256.81	1005.61	121.52	215.62	251.24

Nitrogen fixation bacteria can use small amounts of nitrogen from organic sources and are capable of nitrogen fixation process. Activity of these bacteria are linked with increase of nitrogen content in soils (Miletić and Radulović, 2005). Limiting factors for activity of nitrogen fixation bacteria are organic matter and nutrient content (Brookes, 1995). As can be seen from table 3, in most of samples 15 and 30 days after sowing, abundance of tis physiological group of bacteria was lower in control compared to examinated treatments, while after 130 days lower microbial activity was noticed in treatments with 2 and 4 l ha^{-1} of herbicide compared to control.

Tab. 3. Nitrogen fixation bacteria and *Azotobacte*r sp. in soil

Treatments	Nitrogen fixation bacteria (x 10^2 CFU/g)			*Azotobacter* sp. (x 10^2 CFU/g)		
	sampling after sowing					
	15 d	30 d	130 d	15 d	30 d	130 d
Wing P 2 l ha^{-1}	183.14	152.00	44.57	86.80	57.56	96.87
Wing P 4 l ha^{-1}	75.54	251.73	88.34	73.78	55.84	105.45
Wing P 8 l ha^{-1}	94.66	98.72	192.91	54.68	50.68	75.44
control	92.00	150.36	122.53	87.67	67.01	94.30

Diverse effect of herbicides on nitrogen fixation bacteria was also obtained in previous research (Ali *et al.*, 2014). On the other side, number of *Azotobacter* sp., which plays important role for soil fertility (Jarak and Čolo, 2007) was highest at the end of experiment in all treatments. Lowest *Azotobacter* sp. population was detected in treatment with 8 l of Wing P/ha in all times of sampling (Tab. 3). In almost all treatments, number of *Azotobacter* sp. was highest in control. Similar results were obtained by Mohiuddin and Mohammed (2013) in experiments with 2,4-D and Metribuzine. On the other side, study of Mrkovački *et al*. (2002) has shown that herbicides Ro-Neet and Pyramine had no inhibitory impact on the *Azotobacter* sp.
Fungi and actinomycetes have important role in soil ecosystems. Presence of fungi has stimulatory effects on availability of nutrients (Hoorman, 2011), while actinomycetes are responsible for organic matter degradation (Seong *et al*., 2001).

Tab. 4. Fungi and actinomycetes in soil samples

Treatments	Fungi (x 10^3 CFU/g)			Actinomycetes (x 10^3 CFU/g)		
	sampling after sowing					
	15 d	30 d	130 d	15 d	30 d	130 d
Wing P 2 l ha^{-1}	39.95	24.97	6.81	59.00	180.00	17.13
Wing P 4 l ha^{-1}	32.13	23.15	15.45	70.36	231.91	52.24
Wing P 8 l ha^{-1}	52.98	23.18	13.71	56.46	189.00	57.49
control	9.51	26.63	13.73	105.91	274.00	108.00

The presented results showed that in most of samples fungal activity was lowest at the end of experiment (Tab. 4). In all treatments (except of control) Wing P application reduced the number of soil fungi during experiment. After 30 and 130 days of herbicide application, in most of samples, similar fungal number in control compared with treatments was achieved. Diverse results in determination of herbicides impact on fungal population were noticed in previous researches. Chu *et al*. (2008) showed that chlorpyrifos possessed a negative effect on fungal number. In contrast, stimulatory effect on fungi was obtained by Shan *et al*. (2006). On the other side, herbicide application have had inhibitory effect on growth of actinomycetes. Highest values of actinomycetes number was noticed after 30 days from herbicide application (Tab. 4). Similar results were obtained by Sebiomo *et al*. (2011) in experiment with atrazine, glyphosate and paraquat. In research of Hristeva *et al*. (2014) diverse influence of herbicides on actinomycetes population in soil was observed, depends on types of herbicides. Herbicide Wing, tested in this research, showed inhibitory effect on actinomycetes population, which is in agreement with our results.

CONCLUSION

This study has shown that, except of actinomycetes and *Azotobacte*r sp. number, herbicide Wing in most of treatments had no inhibitory effect on abundance of microorganisms in soil under corn. Our results indicate the potential adaptation of soil microorganisms to various Wing concentrations. Further research will be done in order to determinate the adaptation period of each microbial group, as well as potential of microbial population in degradation of herbicide.

ACKNOWLEDGEMENTS

This work was partially supported by the Ministry of education and science of Republic of Serbia (grant number TR 31080).

BIBLIOGRAPHY

Ali, M., Zaid, M. M., Yahya, S. F. (2014): The effects of post-emergence herbicides on soil microflora and nitrogen fixing bacteria in pea field. International Journal of Chemical, Environmental and Biological Sciences, 2(1): 40-42.

Chu, X., Fang, H., Pan, X., Wang, X., Shan, M., Feng, B., Yu, Y. (2008): Degradation of chlorpyrifos alone and in combination with chlorothalonil and their effects on soil microbial populations. Journal of Environmental Sciences, 20: 464-469.

Brookes, P. C. (1995): The use of microbial parameters in monitoring soil pollution by heavy metals. Biology and Fertility of Soils, 19: 269-279.

Chowdhury, A., Pradhan, S., Saha, M., Sanyal, N. (2008): Impact of pesticides on soil microbiological parameters and possible bioremediation strategies. Indian Journal of Microbiology, 48: 114-127.

Duah-Yentumi, S., Johnson, D. B. (1986): Changes to microflora in response to repeated application of some pesticides. Soil Biology and Biochemistry, 18: 629-635.

Egner, H., Riehm, H., Domingo, W. R. (1960): Untersuchungen uber die chemische boden analyse als grundlage fur die beurteilung des nahrstoffzustandes der boden, II: Chemische extractions metoden zu phosphor und kalium bestimmung. Kungliga lantbrukshugskolans annaler 26: 199-215.

Ekschmitt, K., Griffiths, B. S. (1998): Soil biodiversity and its implications for ecosystem functioning in a heterogeneous and variable environment. Applied Soil Ecology, 10: 201-215.

Emurotu, M. O., Anyanwu, C. U. (2016): Effect of atrazine and butachlor on some soil enzymes activities at different concentrations. Pelagia Research Library, 6(2): 9-15.

Grossbard, E. (1976): Effects on the soil microflora, In: Herbicides: Physiology; Biochemistry; Ecology (Audus, L. J., Ed.), Vol. 2, Academic Press, London, pp. 99-148.

Hoorman, J. J. (2011): The role of soil fungus. Agriculture and natural resources, SAG-14-11. The Ohio State University, 1-6.

Hristeva, T., Yanen, M., Kalinova, S., Bozukov, H. (2014): Comparative analysis of some herbicides from amide and dinitroaniline families on the soil microorganisms. Turkish Journal of Agricultural and Natural Sciences Special Issue, 2: 1447-1454.

IAEA (2001): Impact of long term pesticide usage on soil properties using radiotracer techniques. Vienna, IAEA-TECDOC-1248.

Jacobson, C. S., Hjelmso, M. H. (2014): Agricultural soils, pesticides and microbial diversity. Current Opinion in Biotechnology, 27: 15-20.

Jarak, M., Čolo, J. (2007): Mikrobiologija zemljišta. Poljoprivredni fakultet, Novi Sad.

Li, X. Y., Su, Z. C., Li, X., Zhang, C. G., Zhang, H. W. (2010): Assessing the effects of acetochlor on soil fungal communities by DGGE and clone library analysis. Ecotoxicology, 19 (6): 1111-1116.

Miletić, Z., Radulović, Z. (2005): Biogenost organske prostirke različitih šumskih kultura na deposolima REIK “Kolubara”. Šumarstvo, 4: 11-20.

Mohiuddin, M., Mohammed, M. K. (2013): Influence of fungicide (carbendazim) and herbicides (2, 4-D and metribuzin) on non-target beneficial soil microorganisms of rhizospheric soil of tomato crop. Journal of Environmental Science, Toxicology and Food Technology, 5(1): 47-50.

Peper, I. L., Gerba, C. P., Brendencke, J. W. (1995): Environmental microbiology. Academic Press, San Diego, 11-33.

Piotrowska-Seget, Z., Engel, R., Nowak, E., Kozdroj, J. (2008): Successive soil treatment with captan or oxytetracycline affects non-target microorganisms. World Journal of Microbiology and Biotechnology. 24(12): 2843-2848.

Roger, P. A., Simpson, I., Oficial, R., Ardales, S., Jimenez, R. (1994): Effects of pesticides on soil and water microflora and mesofauna in wetland ricefields: a summary of current knowledge and extrapolation to tempera environments. Australian Journal of Experimental Agriculture, 34: 1057-1068.

Sarnaik, S. S., Kanekar, P. P., Raut, V. M., Taware, S. P., Chavan, K.S., Bhadbhade, B. J. (2006): Effect of application of different pesticides to soybean on the soil microflora. Journal of Environmental Biology, 27(2): 423-426.

Sebiomo, A., Ogundero, V. W., Bankole, S. A. (2011): Effect of four herbicides on microbial population, organic matter and dehydrogenase activity. African Journal of Biotechnology, 10(5): 770-778.

Seong, C. N., Choi, J. H., Baik, K. S. (2001): An improved selective isolation of rare actinomycetes from forest soil. The Journal of Microbiology, 39(1): 17-23.

Sethi, S., Gupta, S. (2013): Impact of pesticides and biopesticides on soil microbial biomass carbon. Universal Journal of Environmental Research and Technology, 3(2): 326-330.

Shan, M., Fang, H., Wang, X., Feng, B., Chu, X. Q., Yu, Y. L. (2006): Effect of chlorpyrifos on soil microbial populations and enzyme activities. Journal of Environmental Sciences, 18(1): 4-5.

Singh, J., Singh, D. K. (2005) Bacterial, azotobacter, actinomycetes, and fungal population in soil after diazinon, imidacloprid, and lindane treatments in groundnut (*Arachis hypogaea* L.) fields. Journal of Environmental Science and Health B, 40(5): 785-800.

Walkley, A. (1935): An examination of methods for determining organic carbon and nitrogen in 15 soils. Journal of Agricultural Sciences, 25: 598-609.

Yang, C. L., Sun, T. H., He, W. X., Zhou, Q. X., Chen, S. (2007): Single and joint effects of pesticides and mercury on soil urease. Journal of Environmental Sciences, 19(2): 210-216.

EFFECTS OF A THIRD GENERATION MICROBIAL INOCULANT ON STRAWBERRY GROWN UNDER ORGANIC CONDITIONS*

Virág Mihálka[1], Attila Hüvely[1], Judit Pető[1], Ildikó Király[1]

Original scientific paper

Summary

A three-year experiment was started in March 2016 on strawberry (*Fragaria × ananassa* cv. Joly). The aim of the study is testing the long term effects of a commercial microbial product on the different soil characteristics and on vegetative growth, yield and mineral composition of strawberry plants. The tested microbial inoculant contains seven different bacterial species. Experimental results of two successive seasons indicated, that application of the microbial inoculant resulted in increased vegetative growth and yield (both in number and size of fruits). Statistically significant increases were detected in root fresh and dry weights of treated plants.

Key words: *PGPR (Plant Growth Promoting Rhizobacteria), Microbial inoculants, organic farming, strawberry, Fragaria × ananassa cv. Joly*

INTRODUCTION

There are numerous studies from the last decade showing that soil treatment with certain bacterial species –mostly associated with the rhizosphere– has positive effects on plant condition, growth and yield (for review see Kaymak *et al*., 2010). These bacteria are termed PGPR (plant growth promoting rhizobacteria) in scientific literature.

It has been known for a long time, that certain PGPR species help phosphorous uptake of plants by solubilizing phosphate (for review see Hilda *et al*., 1999) and enhance plant growth through production of phytohormones, like IAA (Olyunina and Shabaev, 1996; Barazani and Friedmann, 1999; Mirza *et al*., 2001), gibberellic acids (Bastian *et al*., 1998; Joo *et al*., 2004), cytokinins (Timmusk *el al*., 1999.; García de Salamone *et al*., 2001). Furthermore, PGPR bacteria might have positive effect on plant resistance against various pathogens (Burdman *et al*., 2000; Berg *et al*., 2009).

---

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina

[1] John von Neumann University, Faculty of Horticulture and Rural Development
H-6000, Hungary, Kecskemét, Mészöly Gyula tér 1-3.
Correspondence: Virág Mihálka, e-mail: mihalka.virag@kfk.kefo.hu

The numerous beneficial effect of PGPR led to the appearance of different commercial microbial products. First single bacterial strains, later on the combination of different beneficial bacteria were applied as soil inoculants (Matics *et al*., 2015).
In the last decade, third generation bacterial products appeared on the market. These products contain different bacteria species with different properties, and provide complex solutions by improving various soil properties, and by having direct effect on plant growth.
There are many microbial inoculants available on the market -promising increased yield, resistance to different pathogens etc.-, but only a few studies were published testing the long term effect and efficiency under organic growing conditions of such products under controlled and well documented conditions.
For reliable application of the combination of different PGPR strains not only the properties of soil, but also the possible interactions between microbes have to be taken into account (Matics *et al*., 2015).
Therefore, we started a series of studies to investigate the long term impact of the application of microbial products on organically grown strawberry plants on the slightly alkaline sandy soil at the experimental garden of John von Neumann University, Kecskemét, Hungary. In the present study, the product Bactofil B-10 (Agrobio) is tested. Results of two successive seasons are summarized below.

MATERIALS AND METHODS

Experimental site, soil treatments

A three-year experiment was started in March of 2016, at the experimental garden of John von Neumann University. The experimental site is located in Kecskemét (Hungary) (46 °54’N; 19 °40’E). The experimental plots were not cultivated for four years prior to the study.
3 kg/nm^2 of organic manure was worked into the soil one month prior to planting. Thereafter, granulated chicken manure fertilizer (Italpollina) was applied in every 2 months.
The applied microbial product (Bactofil B-10, Agrobio) contains seven different species (*Azospirillum lipoferum, Azotobacter vinelandii, Bacillus megaterium, Bacillus circulans, Bacillus subtilis, Pseudomonas fluororescens, Micrococcus roseus*) in a concentration of $5*10^9$ CFU/cm^3.
One plot was treated – according to the recommendation of the producer– with 2 l/ha of microbial inoculant (treated plot) while the other was left untreated (control plot). Bactofil B-10 was diluted with tap water, sprayed on the soil and worked in immediately. Inoculation was executed in 2016 directly before planting, and repeated in March 2017.

Cultivar, planting, culture conditions

Strawberry cultivar *Fragaria* × *ananassa* cv. Joly (Strawberry plant named 'JOLY', 2012) was chosen for the experiments, which according to the producer, provides sufficient yield even under less optimal conditions (e.g. poor soil, less fertilizers). Frigo transplants of 9-13 mm in diameter were planted with a planting density of 70+40*30 cm in tween-rows in March 2016. During the planting process, 100 ml of 5000 x diluted Bactofil B-10 was poured in every planting hole, before planting the transplants.
All culture practices (fertilization, irrigation, weed control) were performed according to the national and EU regulations of organic farming. In 2016 and 2017 no pest control was carried out.

Soil and leaf tests

Soil tests: Samples were taken in 2016 right before applying organic fertilizer. The tests were repeated in the second year of the experiment (March of 2017). 8-8 subsamples from 0-30 cm depth were collected from the control and the treated plots respectively. Soil samples were dried at 40 °C. Air-dry samples were thoroughly homogenized. Soil extracts of air-dry samples were made by standard methods. Main macro- and microelement contents were measured by optical emission spectrometer (ICP-AES method). P, K, Mg, Na, Zn, Cu, Fe, Mn, S contents were calculated in m/m% dry matter.
Leaf tests: 50-50 leaves were collected from the treated and the control plants. Petiole was removed, and then the leaf blade samples were thoroughly washed. Leaf samples were dried at 70 °C. The air-dry samples were thoroughly minced. For elemental studies powdered samples were digested in a microwave device by means of concentrated nitric acid and hydrogen peroxide (Milestone Ethos Plus). Main macro- and microelement contents were measured by optical emission spectrometer (ICP-AES method). Nitrogen content in leaf blades was determined using the Kjeldahl method (Kjeldahl, 1883) after sulphuric acid digestion (FOSS Kjeltec 2300). Macro element (N, P, K, Ca, Mg, Na) contents were calculated in m/m% dry matter, whereas micro-element (Fe, Mn, Zn, Cu, B, Mo) contents were provided in mg/kg dry matter.

Collection of data

In the first growing season (2016) leaves of 10 treated and 5 control plants were removed after harvesting. Leaves were scanned and the size of leaf area per plant was determined using ImageJ win32 softwer (https://imagej.nih.gov/ij/).
In June 2017, six plants from each plot were randomly selected. Plants were excavated and washed carefully until all the excess soil was removed. Plants were compared visually and photographed. Root and foliage fresh weights were measured in the laboratory, thereafter plants were dried at 80 C° to constant weight. Root and foliage dry weights were recorded with 1 decimal place accuracy.

20-20 plats were selected at the beginning of the growing season. Ripened, marketable fruits of those plants were harvested in 3-4 days intervals. Fruits were collected in two categories: 18-25 mm in diameter (normal) and over 25 mm in diameter (extra), then counted and weighted. Number of fruits and cumulative yield per plant were determined.

Statistical analysis

Data were statistically analysed using SPSS Statistics 23 software. Levene test showed equal variances, therefore one-way ANOVA test was applied for the comparison of means, at significance level of 0.05.

RESULTS AND DISCUSSION

Soil analysis

Based on the results of the first (2016) soil tests (see in the 3rd column of Table 1), the sandy soil of the experimental plots was slightly alkaline, it was low in nitrogen and in potassium, and had an extremely low humus level before starting our study.
In March 2017, soil tests were repeated to see if there were any changes in soil properties as the consequence of inoculation with Bactofil B-10. Results are summarized in the 4th and 5th columns of Table 1.

Table 1. Major physical and chemical properties of the soil at the experimental plots in 2016 and 2017

Test	Measure	2016	2017 Control	2017 Treated
pH (H_2O)		7.83	7.93	7.85
pH (KCl)		7.65	7.58	7.51
Soil plasticity according to Arany (K_A)	K_A unit	29	31	32
Total salts	m/m %	< 0.02	< 0.02	< 0.02
$CaCO_3$	m/m %	1.72	2.53	1.8
Organic matter	m/m %	0.679	1.18	1.22
N	mg/kg	< 1	< 1	< 1
P	mg/kg	355	692	499
K	mg/kg	73.4	82.8	72
Mg	mg/kg	83.5	68.9	72.1
Na	mg/kg	< 5	7.53	5.83
Zn	mg/kg	8.12	13.7	11.2
Cu	mg/kg	36.3	75	47.4
Fe	mg/kg	34.5	55.2	55.3
Mn	mg/kg	35.5	37.5	36.8
S	mg/kg	5.33	1.29	1.68

From 2016 to 2017, the organic matter content of soil increased, thanks to the applied organic fertilizers. On the other hand, only minor increase in organic matter content was measured in the soil, treated with PGPR product compared to the control. At the beginning of the 2018, growing season tests will be repeated to see if there are any improvements in soil properties.

Leaf nutrient content

Results of leaf analysis (Table 2) showed increases in N, P, K, Zn and Cu concentrations of leaves under organic growing conditions of strawberry, which is in agreement with the results of several authors, who reported that bioavailability of different macro- and microelements is enhanced by PGPR (Pii *et al.*, 2015). In the investigations of Esitken *et al.* (2009), significant increase in P, Fe, and Zn was detected in the leaves of organically grown strawberry after treatment with selected PGPR strains. The degree of increase was strongly dependent on the applied bacterial strain (strain combination) and on the treatment method. Similarly to the results of Esitken *et al.* (2010), slight increases in the concentrations of the above nutrients were found, except for Fe (Table 2).

Table 2. Macro- and microelement content of leaves of treated and control strawberry plants

	Control	**Treated**
Dry matter content (m/m %)	31.90	31.00
N (m/m %)	1.95	2.12
P (m/m %)	0.35	0.37
K (m/m %)	1.00	1.06
Ca (m/m %)	0.88	0.69
Mg (m/m %)	0.35	0.31
Na (m/m %)	0.002	0.001
Fe (mg/kg)	136.00	83.00
Mn (mg/kg)	60.50	47.90
Zn (mg/kg)	13.70	14.00
Cu (mg/kg)	6.77	6.84
B (mg/kg)	12.50	10.50
Mo (mg/kg)	<0.500	<0.500

Vegetative growth

In the first season, leaf areas of plants grown on the treated plot were significantly higher (in average almost twice as much as that of the control). At the same time, no

statistically significant difference in size and weight of fruits and in yields were detected based on the results of one-way ANOVA tests (Mihálka *et al.*, 2017).
In the second growing season, visible differences in appearance of plants were recorded when randomly selected whole plants were excavated and compared.
Plants in the lower row of Figure 1, are the treated ones, having larger and more complex roots.

Figure 1. *Fragaria* × *ananassa* cv. Joly plants treated with Bactofil B-10 (lower row) and control (upper row)

Data analysis of fresh and dry weights of roots, foliages and total plants showed that both fresh and dry weight of the treated plants are higher on average. Fresh and dry weights of roots of the treated plants were significantly higher based on the results of statistical analysis (Table 3).
Results of our study indicate -in agreement with several other reports (Mantelin *et al.* 2004; Kloepper *et al.*, 2007) - that the inoculation with certain PGPR strains results in larger roots and differences in root structure. Increase in dry weight of roots as an effect of inoculation with different PGPR strains in different crops like potato, wheat, canola was reported by several authors (Kloepper *et al.*, 1980; Khalid *et al.*, 2004; Bertrand *et al.*, 2001).

Table 3. Effect of Bactofil B-10 on fresh and dry weights of roots and whole plants of *Fragaria* × *ananassa* cv. Joly in 2017. (Statistically significant differences are marked grey)

		Mean	S.D.	Sig.
Root fresh weight (g)	Control	48.00	13.71	0.002
	Treated	85.83	17.12	
Foliage fresh weight (g)	Control	91.17	37.16	0.613
	Treated	82.83	12.29	
Plant fresh weight (g)	Control	139.17	49.43	0.225
	Treated	168.67	25.94	
Root dry weight (g)	Control	17.07	5.22	0.034
	Treated	25.55	6.63	
Foliage dry weight (g)	Control	28.60	11.71	0.425
	Treated	24.42	3.78	
Plant dry weight (g)	Control	45.67	15.86	0.579
	Treated	49.97	8.92	

Yield parameters

Data in Table 4 show that the number of fruits and yield per plant increased in the treated plants compared to the control. 14-15 % growth in yield was recorded on average.

Similar results were reported by Esitken *et al*. (2010). Their results indicate that inoculation of root, floral or foliar spraying of strawberry plants with selected *Bacillus* and *Pseudomonas* strains have positive effect on growth and yield of organically grown strawberries.

Though not all of the treatment –strain combinations resulted in statistically significant differences, the authors reported yield increase of as high as 30% in case of certain combinations (Esitken *et al*., 2010).

Table 4. Effect of the application of Bactofil B-10 on yield of *Fragaria* × *ananassa* cv. Joly in 2017

			Mean	S.D.
Normal size fruits (18-25 mm)	Yield (g / plant)	Control	17.1	12.1
		Treated	23.0	16.9
		Total	20.1	14.8
	Number of fruits / plant	Control	2.7	1.8
		Treated	4.0	2.8
		Total	3.3	2.4
Extra size fruits (25 mm <)	Yield (g / plant)	Control	147.1	96.1
		Treated	164.5	81.8
		Total	155.8	88.5
	Number of fruits / plant	Control	9.6	5.7
		Treated	10.2	4.1
		Total	9.9	4.9
Total	Yield / plant (g)	Control	164.2	98.8
		Treated	187.5	85.9
		Total	175.9	92.1
	Number of fruits / plant	Control	12.3	6.4
		Treated	14.2	5.6
		Total	13.2	6.0

CONCLUSIONS

Based on the data of the present study, long term application of Bactofil B-10 results in well-developed root system in strawberry plants, which is the prerequisite of a healthy plant. Furthermore, the microbial product has a potential effect on yield and nutrient content increase of strawberry grown under organic conditions. Experiments are continued in 2018. Further investigations on the long term effect of PGPR on the soil properties of alkaline sandy soil and on growth and yield of strawberry are in progress.

ACKNOWLEDGEMENT

This research is supported by **EFOP-3.6.1-16-2016-00006** project. The Project is supported by the Hungarian Government and co-financed by the European Social Fund.

REFERENCES

Barazani, O. Z., and Friedman, J. (1999): Is IAA the major root growth factor secreted from plant-growth-mediating bacteria? Journal of Chemical Ecology (25) 10: 2397-2406.

Bastian F., Cohen, A., Piccoli, P., Luna, V., Bottini, R., Baraldi, R., Bottini, R. (1998): Production of indole-3-acetic acid and gibberellins A1 and A3 by *Acetobacter diazotrophicus* and *Herbaspirillum seropedicae* in chemically-defined culture media. Plant growth regulation (24)1: 7-11.

Berg, G. (2009): Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Applied microbiology and biotechnology (84)1: 11-18.

Bertrand, H., Nalin, R., Bally, R., Cleyet-Marel, J. C. (2001): Isolation and identification of the most efficient plant growth-promoting bacteria associated with canola (*Brassica napus*). Biology and Fertility of Soils 33(2): 152-156.

Burdman, S., Jurkevitch, E., Okon, Y. (2000): Recent advances in the use of plant growth promoting rhizobacteria (PGPR) in agriculture. Microbial interactions in agriculture and forestry (Volume II): 229-250.

Esitken, A., Yildiz, H. E., Ercisli, S., Donmez, M. F., Turan, M., Gunes, A. (2010): Effects of plant growth promoting bacteria (PGPB) on yield, growth and nutrient contents of organically grown strawberry. Scientia Horticulturae 124(1): 62-66.

García de Salamone, I. E., Hynes, R. K., & Nelson, L. M. (2001): Cytokinin production by plant growth promoting rhizobacteria and selected mutants. Canadian Journal of microbiology, 47(5) 404-411.

Joo, G. J., Kim, Y. M., Lee, I. J., Song, K. S., Rhee, I. K. (2004): Growth promotion of red pepper plug seedlings and the production of gibberellins by *Bacillus cereus*, *Bacillus macroides* and *Bacillus pumilus*. Biotechnology letters 26(6): 487-491.

Kaymak, HC. (2010): Potential of PGPR in Agricultural innovations. in: Maheshwari DK (ed.) Plant growth and health promoting bacteria. Microbiology Monographs 18. DOI 10.10076978-3-642-13612-2_3. Springer-Verlag Berlin Heidelberg 45-79.

Khalid, A., Arshad, M., Zahir, Z. A. (2004): Screening plant growth promoting rhizobacteria for improving growth and yield of wheat. Journal of Applied Microbiology 96(3): 473-480.

Kjeldahl, J. G. C. T. (1883): Neue methode zur bestimmung des stickstoffs in organischen körpern. Fresenius' Journal of Analytical Chemistry 22(1): 366-382.

Kloepper, J. W., Schroth, M. N. and Miller, T. D. (1980): Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield. Phytopathology 70(11): 1078-1082.

Kloepper, J. W., Gutiérrez-Estrada, A., McInroy, J. A. (2007): Photoperiod regulates elicitation of growth promotion but not induced resistance by plant growth-promoting rhizobacteria. Can. J. Microbiol. 53 (2):159–167.

Mantelin, S., Touraine, B. (2004): Plant growth promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. Journal of experimental botany, 55(394): 27-34.

Matics, H., Biró, B. (2015): History of soil fertility enhancement with inoculation methods. Journal of Central European Agriculture 16 (2): 231-248.

Mihálka, V., Pető, J., Hüvely, A., Király, I. (2017): Investigation of the effect of a microbial inoculant on the yield and foliage of strawberry. Gradus 3(2): 338-343.

Mirza B. S., Mirza M. S., Bano, A., Malik, K. A. (2007): Coinoculation of chickpea with *Rhizobium* isolates from roots and nodules and phytohormone-producing *Enterobacter* strains. Australian Journal of Experimental Agriculture 47(8): 1008-1015.

Olyunina, L. N. and Shabaev, V. P. (1996): Production of indole-3-acetic acid during growth of the rhizosphere bacteria of the genus *Pseudomonas*. Microbiology 65(6): 709-713.

Pii, Y., Mimmo, T., Tomasi, N., Terzano, R., Cesco, S., & Crecchio, C. (2015): Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. Biology and fertility of soils, 51(4), 403-415.

Strawberry plant named ‘JOLY’ (2012). US patent. US PP23126 P3 https://www.google.com/patents/USPP23126

Timmusk, S., Nicander, B., Granhall, U., Tillberg, E. (1999): Cytokinin production by *Paenibacillus polymyxa*. Soil Biology and Biochemistry, 31(13): 1847-1852.

DISTRIBUTION OF STEM RUST (*Puccinia graminis* f. sp. *tritici*) IN SINOP, TURKEY*

Nilüfer Akci[1], Aziz Karakaya[2]

Original scientific paper

Summary

Stem rust caused by *Puccinia graminis* f. sp. *tritici* is an important pathogen of wheat in Turkey. In 2016, a survey was conducted in Sinop province of Turkey in order to elucidate distribution of stem rust in that region. Thirty one wheat fields in Saraydüzü, Durağan, Boyabat and Gerze districts of Sinop province were surveyed. Stem rust was found in 25 fields. Stem rust was present in 100%, 40%, 78% and 90% of the fields inspected in Boyabat, Gerze, Saraydüzü and Durağan districts, respectively. No disease was observed in 6 fields. Disease severity was assessed with a 1-9 scale. In general, the severity values ranged between 7-9. It appears that stem rust is common in that region and it could be a threat to wheat cultivation. Precautions should be taken to ensure wheat crop safety.

Key words: *Stem rust, Puccinia graminis f. sp. tritici, wheat, Sinop, Turkey*

INTRODUCTION

Stem rust caused by *Puccinia graminis* f. sp. *tritici* is an important pathogen of wheat (*Triticum* spp.) in the world (Bockus *et al*., 2010). Yield losses of 70% or more are possible due to stem rust fungus infection (FAO, 2010). This disease is also common in Turkey. In a study conducted in Turkey, Mert *et al*. (2011) reported that in 2007, 91 (43%) out of 207 inspected wheat fields were infected with stem rust, and in 2008, 61 (25%) out of 242 inspected fields were infected. Stem rust situation in Sinop wheat growing areas is largely unknown. The aim of this study was to elucidate stem rust situation in wheat growing areas of Sinop province, Turkey.

MATERIALS AND METHODS

A survey was carried out in June 2016 in order to find the distribution of stem rust in wheat growing areas in Sinop province located in northern part of Turkey (Figure 1).

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Central Research Institute for Field Crops, Yenimahalle, Ankara.
[2] Ankara University, Faculty of Agriculture, Department of Plant Protection, Dışkapı, Ankara, 06110, Turkey
Correspondence: Aziz Karakaya, Ankara University , Faculty of Agriculture, Department of Plant Protection, Dışkapı, Ankara,, 06110, Turkey.
E-mail: karakaya@agri.ankara.edu.tr

Thirty one wheat fields in Saraydüzü, Durağan, Boyabat and Gerze districts in Sinop province were surveyed. Plants were at the soft dough to ripening stages. For surveying, systematic sampling method was used (Aktaş, 2001). Wheat fields were inspected at every 2-10 km. In each field, at least 100 wheat plants were inspected. Bread type wheat (*Triticum aestivum* L.) was present in all inspected fields. Plants were visually examined at the field. In addition, uredospores and/or teliospores of the fungus were examined under a compound microscope (Bockus *et al*., 2010). A 1-9 scale developed by Saari and Prescott (1975) was used for assessing the severity of disease in these fields.

Figure 1. Location of the Sinop province in Turkey where stem rust survey was carried out.

RESULTS AND DISCUSSION

Stem rust was present in all 7 fields inspected in Boyabat district in Sinop province (Figure 2). The percentages of diseased plants in 7 Boyabat fields were 96%, 37%, 80%, 34%, 28%, 15% and 18%. In Boyabat fields, severity values of stem rust ranged between 7-9 (Table 1) .

Figure 2. Stem rust uredial pustules observed in Boyabat district of Sinop province, Turkey.

In Gerze district, stem rust was found in 2 fields out of 5 fields inspected. The percentages of diseased plants in 2 Gerze fields were 35% and 30%. In Gerze fields, severity value of stem rust was 8 in both fields. In Saraydüzü district, 9 fields were inspected and stem rust was present in 7 fields. The percentages of diseased plants in 7 Saraydüzü fields were 17%, 33%, 38%, 3%, 40%, 15% and 30%. In Saraydüzü fields, severity values of stem rust ranged between 7-8. In Durağan district, 10 fields were inspected and stem rust was found in 9 fields.

Table 1. Percentage of stem rust observed fields in Boyabat, Gerze, Saraydüzü and Durağan districts of Sinop province in 2016. For determination of disease severity, a 1 – 9 scale developed by Saari and Prescott (1975) were used.

Districts of Sinop, Turkey	Percentage of disease observed fields	Disease severity
Boyabat	100	7 – 9
Gerze	40	8
Saraydüzü	78	7 – 8
Durağan	90	7 - 9

The percentages of diseased plants in 9 Durağan fields were 85%, 76%, 68%, 75%, 95%, 35%, 40%, 32% and 41%. In Durağan fields, severity values of stem rust ranged between 7-9. In general, severity values ranged between 7-9 in all diseased fields. Stem rust was present in 100%, 40%, 78% and 90% in fields inspected in Boyabat, Gerze, Saraydüzü and Durağan districts, respectively. Disease severity values were high (up to 9 according to Saari and Prescott (1975) scale). In some fields, the disease was detected in wheat heads and awns.

Stem rust of wheat occurs commonly in Turkey (Mert *et al.*, 2011). *Berberis* species, alternate host for stem rust, with aecia on leaves and other plant parts have also been observed in Sinop province. These could be important in generating new races of the pathogen. Detailed studies are needed regarding race structure of the pathogen in this area. It appears that this heteroecious rust is common in Sinop province of Turkey. Control methods should be implemented. Development of stem rust resistant cultivars is the desirable method of control (Roelfs *et al.*, 1992). Stem rust races occurring in Sinop province of Turkey should be determined and wheat cultivars resistant to dominant races should be developed.

CONCLUSIONS

With this study, stem rust situation in Sinop wheat fields were elucidated. Stem rust was present in majority of the fields. Control methods should be established for stem rust disease in this region.

ACKNOWLEDGEMENT

This study was supported by General Directorate of Agricultural Research and Policies, Turkey (Project No: TAGEM-BS-15/12-01/02-02).

REFERENCES

Aktaş, H. (2001): Önemli hububat hastalıkları ve sürvey yöntemleri. T.C. Tarım ve Köyişleri Bakanlığı. Tarımsal Araştırmalar Genel Müdürlüğü. Bitki Sağlığı Araştırmaları Daire Başkanlığı, 74 s. Ankara.

Bockus, W. W., Bowden, R. L., Hunger, R. M., Morrill, W. L., Murray, T. D., Smiley R. W. (eds.). (2010): Compendium of wheat diseases and pests. 3rd ed. APS Press. Minnesota. 171 p.

FAO. (2010): http://www.fao.org/fileadmin/templates/rust/docs/ug99factsheet.pdf (Access date: 06. 12. 2017).

Mert, Z., Karakaya, A., Düşünceli, F., Akan, K., Çetin, L. (2012): Determination of *Puccinia graminis* f. sp. *tritici* races of wheat in Turkey. Turkish Journal of Agriculture and Forestry, 36: 107-120.

Roelfs, A. P., Singh, R. P., Saari, E. E. (1992): Rust diseases of wheat: Concepts and methods of disease management. Mexico. D.F.: CIMMYT. 81 p.

Saari, E. E., Prescott, J. M. (1975): A scale for appraising the foliar intensity of wheat diseases. Plant Disease Reporter, 59: 77-380.

DETERMINATION OF THE REACTIONS OF SOME TURKISH HULLESS BARLEY LINES TO *Drechslera graminea**

Arzu Çelik Oğuz[1], Aziz Karakaya[1], Namuk Ergün[2]

Original scientific paper

Summary

Barley stripe disease caused by the fungus *Drechslera graminea* (teleomorph: *Pyrenophora graminea*) is an important disease of barley (*Hordeum vulgare* L). Twenty-three Turkish hulless barley lines were evaluated for their resistance status using 3 single spore isolates of *Drechslera graminea*. Kayseri isolate was found as the most virulent isolate. One, 2 and 5 lines showed resistant reactions to Kayseri, Eskişehir and Ankara isolates, respectively. Three, 11 and 3 lines showed intermediate responses to Kayseri, Eskişehir and Ankara isolates, respectively. Sixteen lines exhibited resistant or intermediate responses against at least one of the isolates used. Line #2 showed resistant response to three isolates used. Line #6 showed resistant response to one isolate and showed intermediate response to other two isolates. Line #23 showed intermediate response to all isolates. Hulless barley lines that showed resistant and/or intermediate responses to isolates could be used in breeding programs.

Key words: *Barley stripe, Drechslera graminea, Pyrenophora graminea, hulless barley, disease resistance*

INTRODUCTION

Barley (*Hordeum vulgare* L.) is an important cereal crop in the world. According to grain structure, barley genotypes can be divided into two categories as hulless (naked) or hulled (covered) caryopsis (Duan *et al*., 2015). Hulless trait is governed by the recessive 'nud' gene located in chromosome 7HL of barley (Franckowiak and Konishi, 1997; Kikuchi *et al*., 2003; Yalçın *et al*., 2006; Newman and Newman 2008). Barley is mainly used as an animal feed and in malt industry (Geçit, 2016). Hulless barley, in addition to its use as animal feed and in malt industry, could also be used as human food and in food industry (Karaduman, 2006; Yalçın *et al*., 2006; Newman and Newman, 2008). Hulless barley genotypes contain adequate or superior amounts of energy, protein, starch and beta-glucan and have good malt

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Ankara University, Faculty of Agriculture, Department of Plant Protection, Dışkapı, Ankara, 06110, Turkey
[2] Central Research Institute for Field Crops, Yenimahalle, Ankara, Turkey
Correspondence: Aziz Karakaya, Ankara University , Faculty of Agriculture, Department of Plant Protection, Dışkapı, Ankara,, 06110, Turkey.
E-mail: karakaya@agri.ankara.edu.tr

production abilities (Yalçın and Çelik, 2006; Ballestros and Piendl, 1977; Singh and Sosulski, 1985).

Barley stripe disease caused by the fungus *Pyrenophora graminea* (S. Ito & Kurib.) (Anamorphic stage: *Drechslera graminea* (Rabenh. ex Schlecht.) Shoemaker (=*Helminthosporium gramineum* Rabh.) is an important disease of barley worldwide. This disease could be more problematic in areas when diseased barley seeds are used or fungicide application to seed is not practiced. Large losses can occur in severely affected fields (Mathre, 1982). In Turkey, 10%-15% yield losses due to this disease have been reported (Aktaş, 1984). The disease was present in 40% of the barley fields inspected in Central Anatolia Region of Turkey (Karakaya *et al*., 2016). Barley stripe is seed transmitted and it is a one cycle disease. Use of clean seed and seed treatment with fungicides are important disease control measures. The use of genetically resistant genotypes is among the most efficient and economically feasible control methods. Gerlegiz *et al*. (2015) determined the seedling resistance status of some hulled barley and hulless barley cultivars and genotypes to barley net blotch pathogen. However, there is no study related the resistance status of Turkish hulless barley cultivars and genotypes to *Drechslera graminea*. In this study, we evaluated the leaf stripe disease response of 23 Turkish hulless barley lines to three isolates of *Drechslera graminea* collected from different provinces of Turkey.

MATERIALS AND METHODS

Plant materials

Diseased barley plants were collected from Kayseri, Eskişehir and Ankara provinces of Turkey. Significant amount of barley is grown in these provinces. Four, 4 and 2 diseased plants were obtained from randomly selected 9, 7 and 7 barley growing areas of Kayseri, Eskişehir and Ankara provinces, respectively. Seeds of 23 Turkish hulless barley lines were obtained from Barley Breeding Unit of Central Research Institute for Field Crops, Ankara, Turkey.

Isolation of *Drechslera graminea*

Leaves showing barley stripe symptoms were cut into small pieces and surface sterilized using 1% NaOCl solution for 1 minute and rinsed with sterile water. After this step, leaf pieces were placed onto Petri plates containing sterile moist filter. After sporulation of the fungus, single spores were taken under a stereomicroscope and transferred to Potato Dextrose Agar (PDA) plates. Three *D. graminea* isolates selected randomly from each province were used in this experiment.

Inoculation of *Drechslera graminea* isolates

For inoculation of the seeds, sandwich method was used (Mohammad and Mahmood, 1974). Barley seeds were surface sterilized for 3 minutes with 1% NaOCl and after this step they were rinsed with sterile water. Fifteen hulless barley seeds were placed on PDA containing 10 day old *D. graminea* cultures and a sandwich was formed. Three replications were employed. These Petri plates were maintained under room temperature for 3 days. After this period, Petri plates were incubated at 4°C for additional 5-7 days.

Assessment of the reaction of barley lines and virulence of isolates

Using sterile forceps, germinating seeds were placed into 18 cm in diameter pots containing sand, animal manure, and soil (v:v:v 1:1:3). Plants were maintained under greenhouse conditions with a 10─22 ± 3°C night/day temperature regime (Çelik *et al*., 2016). Disease assessments were accomplished 60 days later using a 1-3 scale developed by Tekauz (1983). In this scale, values were classified as followed: 1: Resistant (infection of plants less than 5%) = R, 2: Intermediate (infection of plants between 5%-17%) = I, 3: Susceptible (more than 17% plants infected) = S. Percentage of infection was defined as the number of infected genotypes / total number of genotypes × 100. Analysis of variance was performed for virulence differences among the fungal isolates and means were separated using LSD test (MSTAT, Michigan State University, East Lansing, Michigan, USA).

RESULTS AND DISCUSSION

Disease symptoms started to appear in susceptible genotypes numbered as 12, 18 and 19 after third week. Disease evaluations were done after 60 days. Reactions of barley lines to three *D. graminea* isolates ranged between resistant and susceptible. Pathogenicity differences among the isolates were also evident (Table 1).

Table 1. Reactions of 23 hulless barley lines to 3 different isolates of *Drechslera graminea* 60 days after inoculation. For evaluation, a 1-3 scale developed by Tekauz (1983) was used (R: Resistant, I: Intermediate; S: Susceptible).

Hulless barley lines	Isolates					
	Kayseri		Eskişehir		Ankara	
	Mean disease percent	Scale value	Mean disease percent	Scale value	Mean disease percent	Scale value
1	66.6	3 (S)	14.2	2 (I)	22.2	3 (S)
2	0	1 (R)	0	1 (R)	0	1 (R)
3	50	3 (S)	12.5	2 (I)	25	3 (S)
4	33.3	3 (S)	21.4	3 (S)	17.6	3 (S)
5	66.6	3 (S)	11.7	2 (I)	33.3	3 (S)
6	14.2	2 (I)	16.6	2 (I)	0	1 (R)
7	7.6	2 (I)	4	1 (R)	26.6	3 (S)
8	50	3 (S)	15.3	2 (I)	50	3 (S)
9	33.3	3 (S)	12.5	2 (I)	37.5	3 (S)
10	33.3	3 (S)	14.2	2 (I)	0	1 (R)
11	33.3	3 (S)	12.5	2 (I)	20	3 (S)
12	100	3 (S)	33.3	3 (S)	25	3 (S)
13	50	3 (S)	14.2	2 (I)	33.3	3 (S)
14	50	3 (S)	17.6	3 (S)	25	3 (S)
15	77.7	3 (S)	19	3 (S)	4.5	1 (R)
16	50	3 (S)	21.4	3 (S)	16.6	2 (I)
17	50	3 (S)	28.5	3 (S)	25	3 (S)
18	60	3 (S)	42.8	3 (S)	33.3	3 (S)
19	75	3 (S)	33.3	3 (S)	50	3 (S)
20	71.4	3 (S)	50	3 (S)	8.3	2 (I)
21	25	3 (S)	16.6	2 (I)	0	1 (R)
22	63.6	3 (S)	35.2	3 (S)	31.2	3 (S)
23	10	2 (I)	16.6	2 (I)	9.0	2 (I)
Mean*	46.56±5.23a		20.15±2.49b		21.45±3.14b	

*Means not connected by same letter are significantly different (LSD: 14.23) ($P<0.01$).

Hulless barley line 2 showed resistant reaction to 3 isolates. Line 6, showed resistant reaction to 1 isolate and showed intermediate reaction to 2 isolates. Line 7, line 10 and line 21 showed resistant reaction to 1 isolate and showed intermediate reaction to 1 isolate. Line 23 showed intermediate reaction to 3 isolates. Sixteen lines showed resistant or intermediate reactions to at least one isolate. Isolate obtained from Kayseri was significantly ($P<0.01$) more virulent (46.5% of the plants diseased) than isolates obtained from Ankara (21.4% of the plants diseased) and Eskişehir (18.1% of the plants diseased). The current study is the first one related to resistance status of hulless barley lines in Turkey.

There are limited studies regarding disease resistance of hulless barley genotypes in Turkey. Gerlegiz *et al*. (2015) determined the seedling resistance status of three barley cultivars, one hulless barley cultivar, two candidate hulless barley lines and nine hulless barley genotypes to ten isolates of *Drechslera teres* f. *maculata* under greenhouse conditions. Differences among the reactions of the cultivars and genotypes to the isolates of the fungus were observed. Generally, resistance was found among the hulless barley cultivars and genotypes to *Drechslera teres* f. *maculata*. In this study, virulence differences among the isolates were observed. Balcı *et al*. (2017) evaluated the seedling reactions of 2 hulless barley cultivars, one hulless barley cultivar candidate and 19 hulless barley genotypes to two isolates of spot blotch agent *Cochliobolus sativus* under greenhouse conditions. Hulless barley cultivars Yalın and Özen showed intermediate infection responses. The reactions of hulless barley genotypes ranged between intermediate infection response and high infection response. Hulless cultivar candidate showed low infection response. Also in this study, virulence difference between the isolates was evident.

In Turkey, several studies were performed related to resistance status of barley landraces and cultivars to *Drechslera graminea* isolates. Ulus and Karakaya (2007) determined resistance status of 15 barley cultivars grown in Turkey to 5 isolates of *Drechslera graminea*. Differences among the reactions of the cultivars to the isolates were evident. Barley cultivars Çumra 2001 and Yerçil 147 were resistant to all five isolates. Virulence differences among the isolates were observed. In a study performed by Bayraktar and Akan (2012), barley cultivars Durusu, Balkan 96 (Igri), Çumra 2001 and Anadolu 98 were found as resistant to 13 *Pyrenophora graminea* isolates tested. The authors reported homogenous nature of Turkish isolates. Under greenhouse conditions. Çelik *et al.* (2016) evaluated the reactions of 20 barley landraces and three cultivars of barley to 10 *Drechslera graminea* isolates. Phenotypic variation to leaf stripe disease was observed in the responses of landraces and cultivars of barley with the same and different isolates of the fungus. One barley landrace showed resistance to eight isolates of the fungus. For this landrace, an intermediate response was observed with the remaining two isolates. Cultivar Çumra 2001 showed a resistant reaction to all isolates. Virulence differences were observed among the fungal isolates.

In the current study, reaction differences among the hulless barley lines were observed. Virulence differences among the isolates were also evident. Hulless barley lines showing resistant and/or intermediate reactions to *Drechslera graminea* isolates were found in the current study.

CONCLUSIONS

With this study, for the first time in Turkey, reactions of some hulless barley lines to *Drechslera graminea* isolates were determined. It is found that some hulless barley lines showed resistant and ⁄or intermediate reactions to barley stripe disease. These lines could be used in breeding programs. Barley stripe could be an important disease if precautions are not taken. Use of disease resistant cultivars and lines will be environmentally sound and profitable method for the farmers.

REFERENCES

Aktaş, H. (1984): Spread of leaf spots in barley growing areas of Turkey. Proc. 6th Congr. Un. Phytopth. Mediterr. Cairo, Egypt. 338-341.

Balcı, S., Karakaya, A., Çelik Oğuz, A., Ergün, N., Sayim, İ., Aydoğan, S. (2017): Assessment of the seedling reactions of some hulless barley cultivars and genotypes to *Cochliobolus* leaf spot disease. ICAFOF-International Conference on Agriculture, Forest, Food Sciences and Technologies. 15-17 May 2017. Cappadocia. Turkey.

Ballestros, G., Piendl, A. (1977): Effect of malting on proteolytic, amylolytic and phenolic properties of a malting diastase-rich feed and naked barley. Brewer's Digest, 17: 36-40.

Bayraktar, H., Akan K. (2012): Genetic characterization of *Pyrenophora graminea* isolates and the reactions of some barley cultivars to leaf stripe disease under greenhouse conditions. Turkish Journal Agriculture and Forestry, 36: 329-339.

Çelik, Y., Karakaya, A., Çelik Oğuz, A., Mert, Z., Akan, K., Ergün, N., Sayim, İ. (2016): Determination of the reactions of some barley landraces and cultivars to *Drechslera graminea*. Mediterranean Agricultural Sciences, 29(2): 43-47.

Duan, R., Xiong, H., Wang, A., Chen, G. (2015): Molecular mechanisms underlying hull-caryopsis adhesion/separation revealed by comparative transcriptomic analysis of covered/naked barley (*Hordeum vulgare* L.). Int. J. Mol. Sci., 16: 14181-14193.

Franckowiack, J., Konishi, T. (1997): Naked caryopsis. Barley Genet. Newsletter, 26: 51–52.

Geçit, H. H. (2016): Serin İklim Tahılları (Buğday, Arpa, Yulaf, Triticale). Ankara Üniversitesi Ziraat Fakültesi Yayınları, Yayın No:1640. Ankara.

Gerlegiz, E. T., Karakaya, A., Çelik Oğuz, A., Mert, Z., Sayim, İ., Ergün, N., Aydoğan, S. (2014): Assessment of the seedling reactions of some hulless barley genotypes to *Drechslera teres* f. *maculata*. Selcuk Journal of Agriculture and Food Sciences, 28(2): 63-68.

Karaduman, Y. (2006): Kavuzsuz arpa potansiyeli. Unlu Mamuller Teknolojisi, 74: 21-26.

Karakaya, A., Mert, Z., Çelik Oğuz, A., Çetin, L. (2016): Distribution of barley stripe disease in Central Anatolia, Turkey. Selcuk Journal of Agriculture and Food Sciences, 30(2): 59-61.

Kikuchi, S., Taketa, S., Ichii, M., Kawasaki, S. (2003): Efficient fine mapping of the naked caryopsis gene (nud) by HEGS (High Efficiency Genome Scanning)/AFLP in barley. Theor. Appl. Genet., 108: 73–78.

Mathre, D. E. (Ed.). (1982): Compendium of barley diseases. APS Press. Minnesota, 78 pp.

Mohammad, A., Mahmood, M. (1974): Inoculation techniques in Helminthosporium stripe of barley. Plant Disease Reporter, 58: 32-34.

Newman, C. W., Newman, R. K. (2008): Barley for Food and Health: Science, Technology, and Products. Wiley. New Jersey. 246 pp.

Singh, T., Sosulski, F. W. (1985): Malting of hulless barley cultivars and Glenlea (*Triticum aestivum*) utility wheat. Journal of Food Science, 50: 342-347.

Ulus, C., Karakaya, A. (2007): Assessment of the seedling reactions of some Turkish barley cultivars to barley stripe. Tarım Bilimleri Dergisi, 13: 409-412.

Yalçın, E., Çelik, S. (2006): Kavuzsuz arpa proteinlerinin bazı fonksiyonel özelliklerinin incelenmesi, Türkiye 9. Gıda Kongresi, 24-26 Mayıs 2006, Bolu.

Yalçın, E., Çelik, S., Akar, T., Sayim, İ., Köksel, H. (2006): Kavuzsuz arpanın önemi, beta- glukan ve besin lif içeriği. Hububat Ürünleri Teknolojisi Kongresi Bildiri Kitapçığı, Gaziantep. s. 399-403.

EVALUATION OF THE REACTIONS OF SOME HULLESS BARLEY POPULATIONS TO FOLIAR DISEASES UNDER FIELD CONDITIONS*

Arzu Çelik Oğuz[1], Aziz Karakaya[1], İsmail Sayim[2]

Original scientific paper

Summary

Sixty-five hulless barley F5 populations obtained from ICARDA were examined for foliar diseases at adult stages under field conditions in Ankara, Turkey. Each population contained about 25-30 single heads. From 65 populations, a total of 1818 hulless single head barleys were planted. These plants were evaluated for their resistance status to foliar diseases of barley occurring under natural conditions. A number of diseases were found affecting the populations. Net form of net blotch (*Pyrenophora teres* f. *teres*), spot blotch *(Cochliobolus sativus)*, scald *(Rhynchosporium secalis)*, powdery mildew (*Erysiphe graminis* f. sp. *hordei*), yellow rust (*Puccinia striiformis*), brown rust (*Puccinia hordei*) and stem rust (*Puccinia graminis* f. sp. *tritici*) were observed. Spot blotch, scald, brown rust and yellow rust were present in majority of the populations. Among the populations, the incidence values (number of plants infected of those examined) of net blotch, spot blotch, scald, powdery mildew, yellow rust, brown rust and stem rust ranged between 3.4-23.3%, 3.5-100%, 4.6-88.8, 2.3-12.5%, 2.3-87.5%, 2.5-94.4% and 5.5-6.6%, respectively. A 1-9 scale was used for determining disease severity of all diseases evaluated. Populations 33, 35 and 42 were found to be 96.4%, 96% and 92.8% disease free, respectively. Disease resistant lines can be used as a source of resistance in future breeding studies.

Key words: *Hulless barley, foliar diseases, disease resistance*

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the oldest cultured plants in the world. In Turkey, it is the second most commonly grown cereal after wheat. Turkey is an important gene center of barley (Harlan, 1979; Kün, 1996).

---

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Ankara University, Faculty of Agriculture, Department of Plant Protection, Dışkapı, Ankara, 06110, Turkey
[2] Central Research Institute for Field Crops, Yenimahalle, Ankara, Turkey
Correspondence: Aziz Karakaya, Ankara University , Faculty of Agriculture, Department of Plant Protection, Dışkapı, Ankara,, 06110, Turkey
E-mail: karakaya@agri.ankara.edu.tr

Barley is used mainly as a feed crop and in malt industry (Kün, 1996). Hulless barley or naked barley (*Hordeum vulgare* var. *nudum*) is a form of cultured barley. Hulless barley is also used a feed crop and in malt industry. In addition, hulless barley is used as human food and in food industry. It has a rich beta glucan content and it has high amounts of soluble and unsoluble fibers (Bhatty, 1999; Yalçın *et al*., 2006; Newman and Newman, 2008; Bhatty, 2011).

Barley is affected by a number of diseases. These diseases lower the quantity and quality of barley. These diseases include net form of net blotch caused by *Drechslera teres* f. *teres*, spot form of net blotch caused by *Drechslera teres* f. *maculata*, barley stripe caused by *Drechslera graminea*, brown rust caused by *Puccinia hordei*, stem rust caused by *Puccinia graminis* f. sp. *tritici*, yellow rust caused by *Puccinia striiformis*, scald caused by *Rhynchosporium secalis* (=*Rhynchosporium commune* (Zaffarano *et al*., 2011), spot blotch caused by *Cochliobolus sativus* and powdery mildew caused by *Erysiphe graminis* f. sp. *hordei* (Mathre, 1982).

Genetically, in F5 populations homozygosity was achieved in sufficient levels. In this study, sixty-five hulless barley F5 populations obtained from ICARDA were examined for foliar diseases at adult stages under field conditions in Ankara, Turkey.

MATERIALS AND METHODS

Sixty-five hulless barley F5 populations obtained from ICARDA (International Center for Agricultural Research in the Dry Areas) were examined for foliar diseases at adult stages under field conditions in Ankara, Turkey. Each population contained about 25-30 single heads that were selected randomly in 2014. From 65 populations, a total of 1818 hulless single head barleys were planted in 2015-2016 growing season. Hand plantings of each single head to 1 meter rows were accomplished on October 15, 2015. These plants were evaluated for their resistance status to foliar diseases of barley occurring under natural conditions. Phosphorus (70 kg P_2O_5/ha) and half of the nitrogen (35 kg N/ha) were applied to the seeding depth of soil as diammonium phosphate during seeding. The other half of the nitrogen was applied to soil as 33% ammonium nitrate (35 kg N/ha) with a machine during tillering stage in April. Weeds were removed by hoeing. Disease evaluations were performed during late May and early June, 2016. Plants were inspected macroscopically for foliar diseases at the soft dough to ripening stages. Diagnosis was accomplished in the field. In some cases, samples were examined in the laboratory using a stereomicroscope and a compound microscope. In limited cases, isolations were made to Potato Dextrose Agar medium and cultures were established and fungal propagules were examined. Conidia and conidiophores were also examined after placing the leaves on blotter following surface sterilization with 1% NaOCl. For pathogen identification, Mathre (1982), Niks *et al*. (1989) and Zaffarano *et al*. (2011) were used. Disease severity was assessed with a 1-9 scale developed by Saari and Prescott (1975).

RESULTS AND DISCUSSION

A number of foliar diseases affecting the 65 hulless barley F5 populations obtained from ICARDA were found. Net form of net blotch (*Pyrenophora teres* f. *teres*, anamorph: *Drechslera teres* f. *teres*), spot blotch (*Cochliobolus sativus*, anamorph: *Bipolaris sorokiniana*), scald (*Rhynchosporium secalis*), powdery mildew (*Erysiphe graminis* f. sp. *hordei*), yellow rust (*Puccinia striiformis*), brown rust (*Puccinia hordei*) and stem rust (*Puccinia graminis* f. sp. *tritici*) were observed among the populations (Table 1). Spot blotch, scald, brown rust and yellow rust were present in majority of the populations.

Table 1. Reactions of 65 hulless barley F5 populations to foliar diseases under field conditions in Ankara, Turkey. For assessment of disease severity, a 1-9 scale developed by Saari and Prescott (1975) was used.

Disease	Incidence values (%)	Total number of populations with disease	Severity values	Disease free populations
Net form of net blotch (*Pyrenophora teres* f. *teres*)	3.4-23.3	17	1-7	1, 2, 4, 5, 8, 9, 12, 13, 14, 15, 16, 17, 18, 23, 24, 25, 41
Spot blotch (*Cochliobolus sativus*)	3.5-100	57	1-9	35, 41, 42, 43, 47, 54, 63, 64
Scald (*Rhynchosporium secalis*)	4.6-88.8	54	1-7	6, 7, 11, 12, 26, 33, 34, 35, 42, 46, 61
Powdery mildew (*Erysiphe graminis* f. sp. *hordei*)	2.3-12.5	21	1-5	4, 13, 14, 15, 17, 18, 19, 27, 30, 35, 36, 40, 41, 42, 43, 53, 57, 58, 60, 62
Yellow rust (*Puccinia striiformis*)	2.3-87.5	37	3-7	5; 6, 7, 8, 10, 11, 12, 13, 17, 19, 25, 29, 32, 33, 34, 35, 36, 38, 39, 40, 42, 45, 47, 52, 61, 62, 63, 65
Brown rust (*Puccinia hordei*)	2.5-94.4	47	1-7	5, 6, 7, 11, 12, 13, 20, 25, 26, 34, 35, 37, 38, 42, 46, 50, 57, 58
Stem rust (*Puccinia graminis* f. sp. *tritici*)	5.5-6.6	4	1-5	1, 4, 57, 62

Among the populations, the incidence values (number of plants infected of those examined) of net form of net blotch, spot blotch, scald, powdery mildew, yellow rust, brown rust and stem rust ranged between 3.4-23.3%, 3.5-100%, 4.6-88.8%, 2.3-12.5%, 2.3-87.5%, 2.5-94.4% and 5.5-6.6%, respectively. In populations numbered 4, 9, 10, 11, 15, 20, 23, 24, 25, 28, 30, 38, 46, 49, 50, 51, 53, 54, 55, 56, 57, 58, 59, 60, 61 and 65 all plants were diseased. In the populations numbered 11, 20, 38, 46 and 61, at least one disease was 100% present. Populations 33, 35, 42, 34, 40, 26, 32, 13, 41, 14, 63, 36, 27 and 43 were found to be 96.4%, 96%, 92.8%, 84%, 70%, 50%, 47%, 40%, 36%, 35%, 33%, 32%, 28% and 27% disease free, respectively. In other populations, percent disease free plant numbers were lower. In populations numbered 35 and 42 only powdery mildew disease was present. In population numbered 34 only spot blotch disease and in population numbered 33 only brown rust and spot blotch diseases were observed. Disease resistant lines can be used as a source of resistance in future breeding studies.

Spot blotch caused by the fungus *Cochliobolus sativus* was present in 57 populations. This disease was the most encountered disease among the populations. Percentage of spot blotch diseased plants ranged between 3.5%-100% among the populations. The severity values of spot blotch ranged between 1-9. *Cochliobolus sativus* was found in majority of populations except populations numbered 35, 41, 42, 43, 47, 54, 63 and 64.

Barley scald caused by *Rhynchosporium secalis* was observed in 54 populations. Percentage of scald diseased plants ranged between 4.6%-88.8% among the populations. The severity values of scald ranged between 1-7. *Rhynchosporium secalis* was found in majority of the populations. *Rhynchosporium secalis* was not observed in populations numbered 6, 7, 11, 12, 26, 33, 34, 35, 42, 46 and 61.

Brown rust disease caused by *Puccinia hordei* was observed in 47 populations. Percentage of brown rust diseased plants ranged between 2.5%-94.4% among the populations. The severity values of brown rust ranged between 1-7. *Puccinia hordei* was also common in populations. *Puccinia hordei* was not observed in populations numbered 5, 6, 7, 11, 12, 13, 20, 25, 26, 34, 35, 37, 38, 42, 46, 50, 57 and 58.

Powdery mildew caused by *Erysiphe graminis* f. sp. *hordei* was observed in 21 populations. Percentage of powdery mildew diseased plants ranged between 2.3%-12.5% among the populations. The severity values of powdery mildew ranged between 1-5. *Erysiphe graminis* f. sp. *hordei* was found in populations numbered 1, 4, 13, 14, 15, 17, 18, 19, 27, 30, 35, 36, 40, 41, 42, 43, 53, 57, 58, 60 and 62.

Net form of net blotch caused by *Pyrenophora teres* f. *teres* was observed in 17 populations. Percentage of net form of net blotch diseased plants ranged between 3.4%-23.3% among the populations. The severity values of net form of net blotch ranged between 1-7. *Pyrenophora teres* f. *teres* was found in populations numbered 1, 2, 4, 5, 8, 9, 12, 13, 14, 15, 16, 17, 18, 23, 24, 25 and 41.

Yellow rust caused by *Puccinia striiformis* was observed in 37 populations. Percentage of yellow rust diseased plants ranged between 2.3%-87.5% among the populations. The severity values of yellow rust ranged between 3-7. *Puccinia striiformis* was not found in populations numbered 5, 6, 7, 8, 10, 11, 12, 13, 17, 19, 25, 29, 32, 33, 34, 35, 36, 38, 39, 40, 42, 45, 47, 52, 61, 62, 63 and 65.

Stem rust caused by *Puccinia graminis* f. sp. *tritici* was present in 4 populations. This disease was the least encountered disease among the populations. Percentage of stem rust diseased plants ranged between 5.5%-6.6% among the populations. The severity values of stem rust ranged between 1-5. *Puccinia graminis* f. sp. *tritici* was found in populations numbered 1, 4, 57 and 62.

In Turkey, various diseases occurring on barley plants have been reported. Karakaya *et al*. (2016) reported *Drechslera graminea* from barley fields of Central Anatolia region of Turkey. In the current study, no *Drechslera graminea* was present among the populations. In Turkey, *Drechslera teres* and *Rhynchosporium secalis* were common in barley fields (Karakaya *et al*., 2014). In the current study, scald was common among the hulless barley populations, however, only net type of net blotch was found among the populations. In Eskişehir province of Turkey, Çelik and Karakaya (2015) reported both forms of *Drechslera teres*, *Rhynchosporium secalis, Drechslera graminea*, *Erysiphe graminis* f. sp. *hordei*, *Puccinia graminis* f. sp. *tritici* and *Puccinia hordei* causing diseases on barley plants. Except *Drechslera graminea* and *Drechslera teres* f. *maculata*, all of these disease causing agents were found in the current study among the hulless barley populations. In Kırıkkale province of Turkey, Özdemir *et al*. (2017) reported *Drechslera teres* f. *maculata*, *Drechslera teres* f. *teres*, *Drechslera graminea*, *Rhynchosporium secalis*, *Erysiphe graminis* f. sp. *hordei*, *Puccinia striiformis*, *Puccinia hordei* and *Puccinia graminis* f. sp. *tritici* causing diseases in barley fields. In the current study, we did not observe *Drechslera graminea* and *Drechslera teres* f. *maculata* among the hulless barley populations. However, net form of net blotch (*Pyrenophora teres* f. *teres*), scald (*Rhynchosporium secalis*), powdery mildew (*Erysiphe graminis* f. sp. *hordei*), stem rust (*Puccinia graminis* f. sp. *tritici*), brown rust (*Puccinia hordei*) and yellow rust (*Puccinia striiformis*) were found in varying incidences and severities among the hulless barley populations.

In the current study, *Cochliobolus sativus* was the most commonly encountered pathogen among the hulless barley populations. *Cochliobolus sativus* (anamorph: *Bipolaris sorokiniana*) is commonly responsible for root rot in drier areas with *Fusarium* spp. However, this pathogen could be a serious foliar disease in humid areas or in fields that irrigated (Mathre, 1982).

In this study, resistance status of 65 hulless barley F5 populations obtained from ICARDA were determined under field conditions in Ankara, Turkey. In Turkey, there are limited studies related to seedling stage resistance of hulless barley plants to some diseases. Gerlegiz *et al*. (2015) determined the reactions of some hulless barley cultivar and genotypes to *Drechslera teres* f. *maculata* isolates. Balcı *et al*. (2017) reported seedling resistance status of 2 hulless barley cultivars, one hulless barley cultivar candidate and 19 hulless barley genotypes to *Cochliobolus sativus*. Resistant hulless barley genotypes were found in both studies. In the current study, majority of plants in populations numbered 33, 35, 42 and 34 were disease free.

CONCLUSIONS

In this study, resistance status of 65 hulless barley F5 populations obtained from ICARDA were determined under field conditions in Ankara, Turkey. Percentage of diseases occurring on these populations and their severities were determined. The information gathered from this study will be useful in planning suitable control strategies. Resistant lines determined in this study could be used in future breeding sudies.

ACKNOWLEDGEMENT

We thank Undarmaa Ganzorig and Muharrem Kaymak for their help during this study.

REFERENCES

Balcı, S., Karakaya, A., Çelik Oğuz, A., Ergün, N., Sayim, İ., Aydoğan, S. (2017): Assessment of the seedling reactions of some hulless barley cultivars and genotypes to *Cochliobolus* leaf spot disease. International Conference on Agriculture, Forest, Food Sciences and Technologies. 15-17 May, 2017. Cappadocia, Turkey.

Bhatty, R. S. (1999): The potential of hull-less barley. Cereal Chemistry, 76 (5): 589–599.

Bhatty, R. S. (2011): β-glucan and flour yield of hull-less barley. Cereal Chemistry, 76 (2): 314–315.

Çelik, E., Karakaya, A. (2015): Eskişehir ili arpa ekim alanlarında görülen fungal yaprak ve başak hastalıklarının görülme sıklıklarının ve yoğunluklarının belirlenmesi. Bitki Koruma Bülteni, 55 (2): 157-170.

Gerlegiz, E. T., Karakaya, A., Oğuz, A. Ç., Mert, Z., Sayim, İ., Ergün, N., Aydoğan, S. (2014): Assessment of the seedling reactions of some hulless barley genotypes to *Drechslera teres* f. *maculata*. Selcuk Journal of Agriculture and Food Sciences, 28(2): 63-68.

Harlan, J. R. (1979): On the origin of barley. USDA Agricultural Handbook 338, Washington DC, 10-36.

Karakaya, A., Mert, Z., Çelik Oğuz, A., Çetin, L. (2016): Distribution of barley stripe disease in Central Anatolia, Turkey. Selcuk Journal of Agriculture and Food Sciences, 30(2): 58-61.

Karakaya, A., Mert, Z., Oğuz, A. Ç., Azamparsa, M. R., Çelik, E., Akan, K., Çetin, L. (2014): Current status of scald and net blotch diseases of barley in Turkey. IWBLD – 1st International Workshop on Barley Leaf Diseases, Salsomaggiore Terme, Italy, June 3-6, 2014.

Kün, E. (1996): Tahıllar-1 (Serin İklim Tahılları) 3. baskı. Ankara Üniversitesi Ziraat Fakültesi Yayınları, Yayın No :1451, 322 s., Ankara

Mathre D. E. (ed.) (1982): Compendium of barley diseases. APS Press, Minnesota, USA. 78 pp.

Newman, C. W., Newman, R. K. (2008): Barley for Food and Health: Science, Technology, and Products. Wiley. New Jersey. 246 pp.

Niks, R. E., Dekens, R. G., Ommeren, A. (1989): The abnormal morphology of a very virulent Moroccan isolate belonging or related to *Puccinia hordei*. Plant Disease, 73: 28-31.

Saari E. E., Prescott J. M. (1975): A scale for appraising the foliar intensity of wheat diseases. Plant Disease Reporter, 59: 377-380.

Özdemir, H. Y., Karakaya, A., Çelik Oğuz, A. (2017): Kırıkkale ilinde buğday ve arpa ekim alanlarında görülen fungal yaprak hastalıklarının belirlenmesi. Bitki Koruma Bülteni. 57 (2): 89-112.

Yalçın, E., Çelik, S., Akar, T., Sayim, İ., Köksel, H. (2006): Kavuzsuz arpanın önemi, beta- glukan ve besin lif içeriği. Hububat Ürünleri Teknolojisi Kongresi Bildiri Kitapçığı, Gaziantep. s. 399-403.

Zaffarano, P. L., McDonald, B. A., Linde, C. C. (2011): Two new species of *Rhynchosporium*. Mycologia, 103 (1): 195- 202.

LEAF DISEASES OCCURRING ON BARLEY AND WHEAT FIELDS IN ÇUBUK DISTRICT OF ANKARA, TURKEY*

Memet Zeki İlgen[1], Aziz Karakaya[2], Arzu Çelik Oğuz[2]

Original scientific paper

Summary

Leaf diseases occurring on barley and wheat fields in Çubuk district of Ankara, Turkey were determined. Eighteen barley fields and 17 wheat fields were inspected for the leaf diseases in 2016. In barley fields, diseases caused by *Drechslera teres* f. *maculata*, *Drechslera teres* f. *teres*, *Drechslera graminea*, *Rhynchosporium secalis*, *Erysiphe graminis* f. sp. *hordei*, *Puccinia striiformis*, *Puccinia hordei* and *Puccinia graminis* f. sp. *tritici* were found. In wheat fields, diseases caused by *Puccinia striiformis*, *Puccinia recondita* f. sp. *tritici*, *Puccinia graminis* f. sp. *tritici*, *Septoria tritici* and *Pyrenophora tritici-repentis* were found. Prevalence and severity of these diseases were also recorded.

Key words: *Barley diseases, wheat diseases, Çubuk, Ankara, Turkey*

INTRODUCTION

Barley (*Hordeum vulgare* L.) and wheat (*Triticum* spp.) plants are important in the world and Turkish agriculture. In Turkey, wheat and barley production areas were 7.671.945 ha and 2.740.052 in 2016, respectively. Mean yield values for wheat and barley in the same year were 2690 kg/ha and 2450 kg/ha, respectively (TÜİK, 2017). Diseases occurring on wheat and barley plants lower the quality and quantity of the yield (Mathre, 1982; Bockus *et al*., 2010). In this study, leaf diseases of wheat and barley occurring in wheat and barley growing areas of Çubuk district in Ankara province were determined.

MATERIALS AND METHODS

This study was carried out in central region of Çubuk district of Ankara and its eight villages. For surveying, intensive wheat and barley growing areas were selected. Surveys were conducted at the bolting through close to maturation stages. Plants were

---

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Ministry of Food, Agriculture and Livestock, Directorate of Çubuk District of Food, Agriculture and Livestock, Çubuk, Ankara, Turkey
[2] Ankara University, Faculty of Agriculture, Department of Plant Protection, Dışkapı, Ankara, 06110, Turkey
Correspondence: Aziz Karakaya, Ankara University, Faculty of Agriculture, Department of Plant Protection, Dışkapı, Ankara,, 06110, Turkey.
E-mail: karakaya@agri.ankara.edu.tr

inspected at every 1-5 km and samples were taken according to systematic sampling method (Aktaş, 2001).

Eighteen barley fields and 17 wheat fields were inspected. In each field, 100 plants were examined. For determination of mean disease prevalences mean percentages of diseases in all fields were calculated (means of both disease occurring and no disease occuring fields).

Plants were inspected visually at the field. Some samples were taken to laboratory and isolations were made using Potato Dextrose Agar and V8 agar. Fungal propagules were examined under dissecting microscope and compound miscroscope.

For pathogen identification, Mathre (1982), Bockus *et al*. (2010), Niks *et al*. (1989) and Zaffarano *et al*. (2011) were used. For determining disease severity, a 1-9 scale developed by Saari and Prescott (1975) were used.

RESULTS AND DISCUSSION

The following barley diseases were found in Çubuk central district and 8 villages: spot form of net blotch (*Drechslera teres* f. *maculata*), net form of net blotch (*Drechslera teres* f. *teres*), barley stripe (*Drechslera graminea*), barley scald (*Rhynchosporium secalis= Rhynchosporium commune* (Zaffarona *et al*., 2011)), stripe rust (*Puccinia striiformis*), brown rust (*Puccinia hordei*), stem rust (*Puccinia graminis* f. sp. *tritici*) and powdery mildew (*Erysiphe graminis* f. sp. *hordei*) (Table 1). The mean prevalences of these diseases were 2.1%, 5.2%, 0.33%, 24.6%, 1.1%, 1.38%, 5.5%, and 0.83% for spot form of net blotch, net form of net blotch, barley stripe, barley scald, powdery mildew, stripe rust, brown rust and stem rust, respectively. Scald caused by *Rhynchosporium secalis* was the most common barley disease followed by brown rust and both forms of net blotch. *Drechslera teres* f. *maculata* was observed in 8 fields. The percentage of the diseased plants in these fields ranged between 1-10. In all of these fields, severity value of the spot form of net blotch was 5. *Drechslera teres* f. *teres* was also observed in 8 fields. The percentage of the diseased plants in 7 fields ranged between 1-10, however, 70% of the plants were found infected in a field in Demirci village. In these fields, severity values of the net form of net blotch were between 3-7. In five fields, both forms of the net blotch were present. *Drechslera graminea* was detected in two fields. One and 5 percent of the plants were diseased in these fields. *Rhynchosporium secalis* was observed in 13 fields. In six fields, scald incidence was between 2%-5%. In these fields, severity values of the scald were between 5-6. However, in 7 fields scald incidence was between 50%-80%. In these fields, severity values of the scald were between 5-8. Scald was the most commonly encountered barley disease among the barley fields surveyed. *Erysiphe graminis* f. sp. *hordei* was found in 2 fields. The percentage of the diseased plants in these 2 fields was 10 and severity values were 5 in both fields. *Puccinia striiformis* was found in 2 fields. Five and 20 percent of the plants were diseased in these fields with severity values of 5 and 7. *Puccinia hordei* was found in 11 fields. The percentage of the diseased plants in these fields ranged between 1-25 and severity values were between 3 and 6. Brown rust was the second most commonly encountered barley disease in surveyed areas. *Puccinia graminis* f. sp. *tritici* was found in one field of Dumlupınar village. The percentage of the diseased plants in this field was 15 and disease severity value was 6.

Table 1. Leaf diseases observed in barley fields of Çubuk district of Ankara, Turkey. For assessment of disease severity a 1-9 scale developed by Saari and Prescott (1975) was used.

Barley diseases	Mean disease prevalence	Number of fields with disease	Percentage of diseased plants	Severity values
Spot form of net blotch (*Drechslera teres* f. *maculata*)	2.1%	8	1-10	5
Net form of net blotch (*Drechslera teres* f. *teres*)	5.2%	8	1-70	3-7
Barley stripe (*Drechslera graminea*)	0.33%	2	1-5	-
Barley scald (*Rhynchosporium secalis= Rhynchosporium commune*)	24.6%	13	2-80	5-8
Stripe rust (*Puccinia striiformis*)	1.38%	2	5-20	5-7
Brown rust (*Puccinia hordei*)	5.5%	11	1-25	3-6
Stem rust (*Puccinia graminis* f. sp. *tritici*)	0.83%	1	15	6
Powdery mildew (*Erysiphe graminis* f. sp. *hordei*)	1.1%	2	10	5

The following wheat diseases were found in Çubuk central district and 8 villages: stripe rust (*Puccinia striiformis*), brown rust (*Puccinia recondita* f. sp. *tritici*), stem rust (*Puccinia graminis* f. sp. *tritici*), *Septoria tritici* blotch (*Septoria tritici*) and tan spot (*Pyrenophora tritici-repentis*) (Table 2). The mean prevalences of these diseases were 18.2%, 0.29%, 1.12%, 7.9% and 1.6% for stripe rust, brown rust, stem rust, *Septoria tritici* blotch and tan spot, respectively. Stripe rust caused by *Puccinia striiformis* was the most common wheat disease followed by *Septoria tritici* blotch. Stripe rust was found in 12 wheat fields surveyed. The percentage of the diseased plants in these fields ranged between 5-85 and severity values were between 5 and 9.

Brown rust was found in only one field. The percentage of the diseased plants in this field was 5 and disease severity value was 5. Stem rust was found in 3 fields. The percentage of the diseased plants in these fields ranged between 1-15 and severity values were between 5 and 7. *Septoria tritici* blotch was the second most commonly encountered disease in wheat fields. This disease was observed in 5 fields. The percentage of the diseased plants in 3 fields was 5 in each field and 60 in the other 2 fields located in Kargın and Demirci villages. Disease severity values of this disease in these fields ranged between 5 and 7. Tan spot caused by *Pyrenophora tritici-repentis* was observed in 3 fields. The percentage of the diseased plants in these fields ranged between 3-20 and severity values were between 5 and 6.

Table 2. Leaf diseases observed in wheat fields of Çubuk district of Ankara, Turkey. For assessment of disease severity a 1-9 scale developed by Saari and Prescott (1975) was used.

Wheat diseases	Mean disease prevalence	Number of fields with disease	Percentage of diseased plants	Severity values
Stripe rust (*Puccinia striiformis*)	18.2%	12	5-85	5-9
Brown rust (*Puccinia recondita* f. sp. *tritici*)	0.29%	1	5	5
Stem rust (*Puccinia graminis* f. sp. *tritici*)	1.12%	3	1-15	5-7
Septoria tritici blotch (*Septoria tritici*)	7.9%	5	5-60	5-7
Tan spot (*Pyrenophora tritici-repentis*)	1.6%	3	3-20	5-6

Differences in the incidence and prevalences of the diseases were observed. The prevalences of the diseases ranged between 3-9.

These diseases are commonly encountered in Turkey and they are found in barley and wheat growing areas of Turkey with varying intensities (Iren, 1981; Finci, 1982; Mamluk *et al*., 1997; Yıldırım *et al*., 1999; Karakaya *et al*., 2014).

Çelik and Karakaya (2015) determined the leaf diseases of barley occurring barley growing areas of Eskişehir province, Turkey. They found both forms of net blotch (*Drechslera teres*), barley stripe (*D. graminea*), brown rust (*Puccinia hordei*), scald (*Rhynchosporium secalis*), powdery mildew (*Erysiphe graminis* f. sp. *hordei*) and stem rust (*Puccinia graminis* f. sp. *tritici*). They found net blotch and scald as the most common diseases in Eskişehir. In the current study, scald caused by *R. secalis* was found as the most common disease in Çubuk district of Ankara province, Turkey.

Özdemir *et al*. (2017) determined the leaf diseases of barley occurring in barley growing areas of Kırıkkale province, Turkey. They found diseases caused by *Drechslera teres* f. *maculata*, *Drechslera teres* f. *teres*, *Drechslera graminea*, *Rhynchosporium secalis*, *Erysiphe graminis* f. sp. *hordei*, *Puccinia striiformis*, *Puccinia hordei* and *Puccinia graminis* f. sp. *tritici* in barley fields of Kırıkkale. In this study, diseases caused by *Drechslera teres* f. *maculata* and *Rhynchosporium secalis* were found as the most common diseases. In the current study, scald caused by *R. secalis* was found as the most common disease in Çubuk district of Ankara province, Turkey.

Özdemir *et al*. (2017) determined the leaf diseases of wheat occurring in wheat growing areas of Kırıkkale province, Turkey. They found diseases caused by *Erysiphe graminis* f. sp. *tritici*, *Puccinia striiformis*, *Puccinia recondita* f. sp. *tritici*, *Puccinia graminis* f. sp. *tritici*, *Septoria tritici*, *Pyrenophora tritici-repentis* and *Alternaria* sp. in wheat fields of Kırıkkale. In this study, stripe rust caused by *Puccinia striiformis* was found as the most common disease followed by diseases caused by *Puccinia graminis* f. sp. *tritici* and *Septoria tritici*. *Erysiphe graminis* f. sp. *tritici* and *Alternaria* sp. found in their study was not detected in the current study. Stripe was the most commonly encountered disease and incidence of stripe rust was found more in the current study.

It appears that a large number of diseases are present in the barley and wheat growing areas of Çubuk district of Ankara Province, Turkey. Control methods should be implemented regarding these diseases.

CONCLUSIONS

Numerous diseases were present in the barley and wheat growing areas of Çubuk district of Ankara Province, Turkey. Scald caused by *Rhynchosporium secalis* was the most common barley disease followed by brown rust and both forms of net blotch. Stripe rust caused by *Puccinia striiformis* was the most common wheat disease followed by *Septoria tritici* blotch. Control methods should be implemented regarding these diseases.

REFERENCES

Aktaş, H. (2001): Önemli hububat hastalıkları ve sürvey yöntemleri. T.C. Tarım ve Köyişleri Bakanlığı. Tarımsal Araştırmalar Genel Müdürlüğü. Bitki Sağlığı Araştırmaları Daire Başkanlığı,. Ankara. 74 s.

TÜİK. (2017): http://www.tuik.gov.tr/PreTablo.do?alt_id=1001 Access date: 15.05.2017.

Bockus, W. W., Bowden, R. L., Hunger, R. M., Morrill, W. L., Murray, T. D., Smiley, R. W. (eds.). (2010): Compendium of wheat diseases and pests. 3rd ed. APS Press. Minnesota. 171 p.

Çelik, E., Karakaya, A. (2015): Eskişehir ili arpa ekim alanlarında görülen fungal yaprak ve başak hastalıklarının görülme sıklıklarının ve yoğunluklarının belirlenmesi**.** Bitki Koruma Bülteni, 55(2): 157-170.

Finci, S. (1982): Marmara Bölgesinde buğday ekim alanlarında görülen *Septoria* fungusunun türleri, yayılışları ve çeşit reaksiyonları üzerinde çalışmalar. Bitki Koruma Bülteni, 22 (2), 72-88.

İren, S. (1981): Wheat diseases in Turkey. EPPO Bulletin, 11 (2), 47-52.

Karakaya, A., Mert, Z., Oğuz, A. Ç., Azamparsa, M. R., Çelik, E., Akan, K., Çetin, L. (2014): Current status of scald and net blotch diseases of barley in Turkey. IWBLD – 1st International Workshop on Barley Leaf Diseases, Salsomaggiore Terme, Italy, June 3-6, 2014.

Mamluk, O. F., Çetin, L., Braun, H. J., Bolat, N., Bertschinger, L., Makkouk, K. M., Yıldırım, A. F., Saari, E. E., Zencirci, N., Albustan, S., Calı, S., Beniwal, S. P. S., Düşünceli, F. (1997): Current status of wheat and barley diseases in the Central Anatolia Plateau of Turkey. Phytopathologia Mediterranea, 36, 167-181.

Mathre, D. E. (ed.) (1982): Compendium of barley diseases. APS Press, Minnesota, USA. 78 p.

Niks, R. E., Dekens, R. G., Ommeren, A. (1989): The abnormal morphology of a virulent Moroccan isolate belonging or related to *Puccinia hordei*. Plant Disease, 73: 28-31.

Özdemir, H. Y., Karakaya, A., Çelik Oğuz, A. (2017): Kırıkkale ilinde buğday ve arpa ekim alanlarında görülen fungal yaprak hastalıklarının belirlenmesi. Bitki Koruma Bülteni, 57(2): 89 – 112.

Saari, E. E., Prescott, J. M. (1975): A scale for appraising the foliar intensity of wheat diseases. Plant Disease Reporter, 59: 377-380.

Yıldırım, A. F., Kınacı, E., Hekimhan, H., Çeri, S. (1999): Konya, Karaman, Niğde ve Aksaray yörelerinde tahıllarda önemli hastalıkların durumu ve bunlara dayanıklılık kaynaklarının araştırılması. Orta Anadolu’da Hububat Tarımının Sorunları ve Çözüm Yolları Sempozyumu, 8-11 Haziran 1999, Konya, 404-413.

Zaffarano, P. L., McDonald, B. A., Linde, C. C. (2011): Two new species of *Rhynchosporium*. Mycologia, 103 (1): 195- 202.

DETERMINATION OF THE REACTIONS OF IRANIAN BARLEY LANDRACE POPULATIONS TO FOLIAR DISEASES UNDER FIELD CONDITIONS*

Aziz Karakaya[1], Arzu Çelik Oğuz[1], Sinan Aydoğan[2], Amir Rahimi [3]

Original scientific paper

Summary

Twenty two barley landrace populations obtained from different regions of northwest Iran were evaluated for their resistance status to foliar diseases under field conditions in Ankara, Turkey. Landrace populations exhibited differences in terms of disease resistance. Scald caused by *Rhynchosporium secalis*, yellow rust caused by *Puccinia striiformis* and brown rust caused by *Puccinia hordei* were the most common diseases affecting barley landrace populations. Net form of net blotch caused by *Drechslera teres* f. *teres* was present in 6 populations. The percentage of diseased plants ranged between 5%-57% among these populations. Scald caused by *Rhynchosporium secalis* was present in all 22 populations examined. The percentage of diseased plants ranged between 57%-100% among these populations. In eight populations all plants were found infected with *R. secalis*. Yellow rust caused by *Puccinia striiformis* was present in 21 populations. The percentage of diseased plants ranged between 4.7%-100% among these populations. Brown rust caused by *Puccinia hordei* was present in 21 populations. The percentage of diseased plants ranged between 11%-86% among these populations. Stem rust caused by *Puccinia graminis* f. sp. *tritici* was present in 4 populations. The percentage of diseased plants ranged between 4%-16% among these populations. Powdery mildew caused by *Erysiphe graminis* f. sp. *hordei* was present in one population. The percentage of diseased plants was 38% in this population. Severity of these diseases was assessed with a 1-9 scale. Severity values for net blotch, scald, powdery mildew and rust diseases ranged between 1-9, 1-9, 3-7 and 5-9, respectively.

Key words: *Barley landraces, foliar diseases, disease resistance*

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Ankara Univ., Faculty of Agricult., Dept. of Plant Prot., Ankara, Turkey
[2] Central Research Institute for Field Crops, Yenimahalle, Ankara, Turkey
[3] Urmia Univ., Faculty of Agricult., Dept. of Agronomy, Urmia, West Azerbaijan, Iran
Correspondence: Aziz Karakaya, Ankara Univ., Fac. of Agricult., Dept. of Plant Prot., Dışkapı, Ankara, Turkey
E- mail: karakaya@agri.ankara.edu.tr

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the most important crops in the world. Barley is used as a feed crop and in malt industry (Geçit, 2016). Barley is domesticated in Fertile Crescent region which includes parts of Iran and Turkey (Ceccarelli and Grando, 2000). This area is an important gene center of barley and this crop is cultivated in this area for a long time. Barley landraces are good source of disease resistance (Yitbarek *et al*., 1998; Ceccarelli and Grando, 1999; Çelik Oğuz *et al*., 2017). Barley landraces are still planted in many regions of the world including Iran and Turkey. Barley is affected by a number of diseases. These diseases include barley scald caused by *Rhynchosporium secalis* (=*Rhynchosporium commune* (Zaffarano *et al*., 2011), net blotch caused by *Drechslera teres*, powdery mildew caused by *Erysiphe graminis* f. sp. *hordei*, barley stripe caused by *Drechslera graminea*, brown rust caused by *Puccinia hordei* and yellow rust caused by *Puccinia striiformis* (Mathre, 1982).

In this study, 22 barley landrace populations obtained from northwest Iran were examined under field conditions in Ankara, Turkey for their response to foliar diseases.

MATERIALS AND METHODS

Four, 3, 4, 11 barley landrace populations were obtained from Oshnaviye, Piranshahr, Bukan and Naghadeh regions of norhwest Iran. Oshnaviye-1 population consisted of 89% 2-rowed landraces and 11% 6-rowed landraces. Oshnaviye-2 and Oshnaviye-3 populations consisted of 6-rowed landraces and Oshnaviye-4 population consisted of 2-rowed landraces. Piranshahr-1 population consisted of 2-rowed cultivars which 13% were dark colored landraces. Piranshahr-2 population consisted of 87% 2-rowed landraces and 13% 6-rowed landraces. Piranshahr-3 population consisted of 6-rowed landraces. Bukan-1 population consisted of 86% 2-rowed landraces and 14% 6-rowed landraces. Bukan-2 population consisted of 80% 2-rowed landraces and 20% 6-rowed landraces. Bukan-3 population consisted of 88% 2-rowed landraces and 12% 6-rowed landraces. Bukan-4 population consisted of 2-rowed landraces. Naghadeh populations consisted of 2-rowed landraces with the exception of Naghadeh-6 population which contained 11% 6-rowed landraces. Naghadeh-4, Naghadeh-6, Naghadeh-8 and Naghadeh 10 populations contained 13%, 22%, 20% and 10% dark colored landraces, respectively.

Seeds obtained from farmers' fields from 4 different regions of northwest Iran (Oshnaviye, Piranshahr, Bukan and Naghadeh) were planted with a sowing machine on October 28, 2015. Length and width of the plots were 5 m and 1.08 m, respectively. There were 6 rows and row spacing between the rows were 18 cm. Approximately 500 seeds per square meter was planted. Phosphorus (70kg P_2O_5/ha) and half of the nitrogen were applied to seeding depth of soil (35kg N/ha) as diammonium phosphate during seeding. In spring the other half of the nitrogen was applied to soil as 33% ammonium nitrate (35kg N/ha) with a machine during tillering stage in April. Weeds were removed by hoeing. Disease evaluations were performed during June, 2016. In July, seeds were harvested and maintained in Gene Bank of Central Research Institute for Field Crops, Ankara, Turkey.

Plants were visually inspected at the plots. For scald, net type of net blotch and powdery mildew diseases, identification was based on assessment of characteristic symptoms in the field. For rust diseases, in addition to characteristic symptoms, morphology of spores were examined under a compound microscope. For pathogen identification, Mathre (1982), Niks *et al*. (1989) and Zaffarano *et al*. (2011) were used. Disease severity was assessed with a 1-9 scale developed by Saari and Prescott (1975).

RESULTS AND DISCUSSION

A number of diseases were found among the barley landrace populations obtained from northwest Iran (Table 1). Scald caused by *Rhynchosporium secalis*, yellow rust (stripe rust) caused by *Puccinia striiformis*, brown rust (leaf rust) caused by *Puccinia hordei*, net form of net blotch caused by *Drechslera teres* f. *teres*, stem rust caused by *Puccinia graminis* f. sp. *tritici* and powdery mildew caused by *Erysiphe graminis* f. sp. *hordei* were present in barley landrace populations (Figure 1). Scald caused by *Rhynchosporium secalis*, yellow rust caused by *Puccinia striiformis* and brown rust caused by *Puccinia hordei* were the most common diseases affecting barley landrace populations.

Scald caused by *Rhynchosporium secalis* was present in all 22 populations examined. The percentage of diseased plants ranged between 57%-100% among these populations. In eight populations all plants were found infected with *R. secalis*. In 4 Oshnaviye region populations the percentage of diseased plants were 57%, 100%, 60% and 100%, respectively. Scald disease severity values in these populations ranged between 5-9, 5-9, 7-9 and 5-7, respectively. In 3 Piranshahr region populations the percentage of diseased plants were 63%, 88% and 90%, respectively. Scald disease severity values in these populations ranged between 5-8, 5-9 and 5-9, respectively. In dark colored Piranshahr-1 population landraces only scald was present (70% of the plants with a severity value 7). In 4 Bukan region populations the percentage of diseased plants were 71%, 100%, 100% and 93%, respectively. Scald disease severity values in these populations ranged between 5-9, 5-9, 7-8 and 5-9, respectively. In 11 Naghadeh region populations the percentage of diseased plants were 89%, 100%, 100, 92%, 100%, 95%, 100%, 98%, 93%, 98% and 95%, respectively. Scald disease severity values in these populations ranged between 7-9, 7-9, 7-9, 1-9, 7-9, 7-9, 8-9, 7-

9, 6-9, 7-9 and 5-9, respectively. This disease was the most commonly encountered disease among the all landrace populations.

Figure 1. Scald (left) and net form of net blotch (right) diseases observed in Iranian landrace populations under field conditions in Ankara, Turkey.

Yellow rust caused by *Puccinia striiformis* was present in 21 populations. The percentage of diseased plants ranged between 4.7%-100% among these populations. In 4 Oshnaviye region populations the percentage of yellow rust infested plants were 11%, 29%, 9% and 10%, respectively. Yellow rust disease severity values in these populations ranged between 5-7, 5-7, 7-9 and 7-9, respectively. In 3 Piranshahr region populations the percentage of yellow rust infested plants were 75%, 63% and 33%, respectively. Yellow rust disease severity values in these populations ranged between 7-9, 5-7 and 7-9, respectively. In 4 Bukan region populations the percentage of yellow rust infested plants were 86%, 30%, 75% and 56%, respectively. Yellow rust disease severity values in these populations ranged between 7-9, 5-9, 7-9 and 7-9, respectively. Yellow rust was not observed in one Naghadeh population. In 10 Naghadeh region populations the percentage of yellow rust infested plants were 89%, 100%, 60%, 5%, 33%, 100%, 100%, 93%, 50% and 21%, respectively. Yellow rust disease severity values in these populations ranged between 7-9, 7-9, 7-9, 5-9, 5-9, 7-9, 7-9, 7-9, 5-9 and 5-9, respectively.

Brown rust caused by *Puccinia hordei* was present in 21 populations. The percentage of diseased plants ranged between 11%-86% among these populations. In Oshnaviye region populations the percentage of brown rust infested plants were 33%, 71%, 64% and 11%, respectively. Brown rust disease severity values in these populations ranged between 5-7, 5-7, 5-7 and 7-9, respectively. In 3 Piranshahr region populations the percentage of brown rust infested plants were 25%, 56% and 22%, respecitvely. Brown rust disease severity values in these populations ranged between 7-9, 5-9 and 7-9, respectively. In 4 Bukan region populations the percentage of brown rust infested plants were 14%, 20%, 86% and 67%, respectively. Brown rust disease severity values in these populations ranged between 5-7, 5-7, 7-9 and 5-9, respectively. Brown rust was not observed in one Naghadeh population. In 10 Naghadeh region populations the percentage of brown rust infested plants were 44%, 33%, 22%, 60%, 75%, 25%, 22%, 13%, 70% and 75%, respectively. Brown rust disease severity values in these populations ranged between 5-9, 7-9, 7-9, 5-9, 5-7, 5-9, 7-9, 7-9, 6-9 and 5-7, respectively.

Stem rust caused by *Puccinia graminis* f. sp. *tritici* was present in 4 populations. The percentage of diseased plants ranged between 4%-16% among these populations. Stem rust was present in one population of Oshnaviye, one population of Piranshahr and 2 populations of Naghadeh. The percentage of stem rust infested plants in these populations were 9%, 13%, 4% and 16%, respectively. Stem rust disease severity values in these populations ranged between 5-7, 7-9, 5-7 and 5-9, respectively.

Net form of net blotch caused by *Drechslera teres* f. *teres* was present in 6 populations. The percentage of diseased plants ranged between 5%-57% among these populations. Net form of net blotch was found in 3 populations of Oshnaviye, one population of Piranshahr, one population of Bukan and one population of Naghadeh. The percentage of net form of net blotch infested plants in these populations were 5%, 14%, 57%, 13%, 9% and 12%, respectively. Net form of net blotch severity values in these populations ranged between 1-5, 5-7, 7-9, 7-9, 5-7 and 7-9, respectively.

Powdery mildew caused by *Erysiphe graminis* f. sp. *hordei* was present in one population obtained from Bukan region. The percentage of diseased plants was 38% in this population. Powdery mildew severity values among the plants of this population ranged between 3-7.

Barley landraces are good sources of disease resistance. In a study performed by Çelik Oğuz *et al*. (2017) novel barley landraces resistant to net and spot forms of net blotch pathogen were identified.

Mert *et al*. (2014), under field conditions, evaluated the resistance status of 200 Turkish barley landraces to *Drechslera teres* and *Rhynchosporium secalis*. Although resistance to net blotch among the Turkish landraces was common only a limited number of landraces exhibited resistance to scald. Also, in the current study, scald was the most commonly encountered disease and resistant landraces were limited. In current study, only net type of net blotch was present and this disease was present in 6 populations.

Table 1. Reactions of Iranian barley landrace populations to foliar diseases under field conditions in Ankara, Turkey. For assessment of disease severity a 1-9 scale developed by Saari and Prescott (1975) was used.

Diseases	Percent disease range among the populations	Oshnaviye region			Piranshahr region			Bukan region			Naghadeh region		
		Number of disease observed populations	Diseased plants (%)	Disease severity	Number of disease observed populations	Diseased plants (%)	Disease severity	Number of disease observed populations	Diseased plants (%)	Disease severity	Number of disease observed populations	Diseased plants (%)	Disease severity
Scald (*R. secalis*)	57-100	4	57 100 60 100	5-9 5-9 7-9 5-7	3	63 88 90	5-8 5-9 5-9	4	71 100 100 93	5-9 5-9 7-8 5-9	11	89 100 100 92 100 95 100 98 93 98 95	7-9 7-9 7-9 1-9 7-9 7-9 8-9 7-9 6-9 7-9 5-9
Yellow rust (*P. striiformis*)	4.7-100	4	11 29 9 10	5-7 5-7 7-9 7-9	3	75 63 33	7-9 5-7 7-9	4	86 30 75 56	7-9 5-9 7-9 7-9	10	89 100 60 5 33 100 100 93 50 21	7-9 7-9 7-9 5-9 5-9 7-9 7-9 7-9 5-9 5-9
Brown rust (*P. hordei*)	11-86	4	33 71 64 11	5-7 5-7 5-7 7-9	3	25 56 22	7-9 5-9 7-9	4	14 20 86 67	5-7 5-7 7-9 5-9	10	44 33 22 60 75 25 22 13 70 75	5-9 7-9 7-9 5-9 5-7 5-9 7-9 7-9 6-9 5-7

Table 1. Reactions of Iranian barley landrace populations to foliar diseases under field conditions in Ankara, Turkey. For assessment of disease severity a 1-9 scale developed by Saari and Prescott (1975) was used (continued).

Diseases	Percent disease range among the populations	Oshnaviye region			Piranshahr region			Bukan region			Naghadeh region		
		Number of disease observed populations	Diseased plants (%)	Disease severity	Number of disease observed populations	Diseased plants (%)	Disease severity	Number of disease observed populations	Diseased plants (%)	Disease severity	Number of disease observed populations	Diseased plants (%)	Disease severity
Stem rust (*P. graminis* f. sp. *tritici*)	4-16	1	9	5-7	1	13	7-9	0			2	4 16	5-7 5-9
Net form of net blotch (*D. teres* f. *teres*	5-57	3	5 14 57	1-5 5-7 7-9	1	13	7-9	1	9	5-7	1	12	7-9
Powdery mildew (*E. graminis* f. sp. *hordei*		0			0			1	38	3-7	0		

Current study identified the diseases occurring in 22 different barley landrace populations obtained from norhwest Iran. Scald, yellow rust and brown rust were the most commonly encountered diseases among these populations. Net form of net blotch, stem rust and powdery mildew diseases were found on 6, 4 and one populations, respectivey. Variation was found among the barley landrace populations in terms of disease resistance. Reactions of the landrace populations showed different resistance responses to different diseases. Scald was the most commonly encountered disease among the populations followed by yellow rust and brown rust. Some diseases were observed only in limited populations.

CONCLUSIONS

Twenty two barley landrace populations obtained from different regions of northwest Iran were evaluated for their resistance status to foliar diseases under field conditions in Ankara, Turkey. Scald caused by *Rhynchosporium secalis*, yellow rust caused by *Puccinia striiformis* and brown rust caused by *Puccinia hordei* were the most common diseases affecting barley landrace populations. To a lesser degree, net form of net blotch, stem rust and powdery mildew diseases were found. Variation was found among the barley landrace populations in terms of disease resistance. The highest scald incidence was found in the Naghadeh population. All plants were found infected with *R. secalis* in four, two and two populations obtained from Naghadeh, Bukan and Oshnaviye regions, respectively. The lowest yellow rust incidence was found in the Piranshahr population. Powdery mildew disease was found in only one population obtained from Bukan region. Generally, disease severity values of diseases were high in populations.

ACKNOWLEDGEMENT

We thank Cansu Doğan and Bahaddin Çelik for their help during this study.

REFERENCES

Ceccarelli, S., Grando, S. (2000): Barley landraces from the Fertile Crescent: A lesson for plant breeders. Pages 51-76 *in* Genes in the field. On-farm conservation of crop diversity (ed. S.B. Brush). International Plant Genetic Resources Institute, Rome. International Development Research Centre, Ottawa. Lewis publishers, Boca Raton.

Çelik Oğuz, A., Karakaya, A., Ergün, N., Sayim, İ. (2017): Turkish barley landraces resistant to net and spot forms of *Pyrenophora teres*. Phytopathologia Mediterranea, 56 (2): 217-223.

Geçit, H. H. (2016): Serin İklim Tahılları (Buğday, Arpa, Yulaf, Triticale). Ankara Üniversitesi Ziraat Fakültesi Yayınları, Yayın No:1640. Ankara.

Mathre, D. E. (ed.) (1982): Compendium of barley diseases. APS Press, Minnesota, USA.

Mert, Z., Karakaya, A., Çelik Oğuz, A., Azamparsa, M. R., Ergün, N., Sayim, İ. (2014): Field evaluation of some Turkish barley landraces to scald and net blotch of barley. IWBLD – 1st International Workshop on Barley Leaf Diseases, Salsomaggiore Terme, Italy, June 3-6, 2014.

Niks, R. E., Dekens, R. G., Ommeren, A. (1989): The abnormal morphology of a very virulent Moroccan isolate belonging or related to *Puccinia hordei*. Plant Disease, 73: 28-31.

Saari E. E., Prescott, J. M. (1975): A scale for appraising the foliar intensity of wheat diseases. Plant Disease Reporter, 59: 377-380.

Yitbarek, S., Berhane, L., Fikadu, A., Van Leur, J. A. G., Grando, S., Ceccarelli, S. (1998): Variation in Ethiopian barley landrace populations for resistance to barley leaf scald and net blotch. *Plant Breeding* 117, 419-423.

Zaffarano, P. L., McDonald, B. A., Linde, C. C. (2011): Two new species of *Rhynchosporium*. Mycologia, 103 (1): 195- 202.

RESPONSE OF IRANIAN BARLEY LANDRACES TO *Drechslera graminea**

Aziz Karakaya[1], Arzu Çelik Oğuz[1], Amir Rahimi[2]

Original scientific paper

Summary

Barley (*Hordeum vulgare* L.) landraces are good source of disease resistance. In this study, twenty-five Iranian barley landraces obtained from different regions of northwest Iran were evaluated for their resistance status to *Drechslera graminea,* the causal agent of the barley stripe disease. Three single spore isolates of the fungus obtained from Kayseri, Eskişehir and Ankara provinces of Turkey were used for inoculation. Virulence differences among the isolates were observed. Mean virulence values of Kayseri, Eskişehir and Ankara isolates were 31.12, 14.74 and 15.95, respectively. Kayseri isolate was the most virulent isolate ($P<0.01$). One, five and six landraces showed resistant reactions to Kayseri, Eskişehir and Ankara isolates, respectively. Six, eleven and eleven landraces showed intermediate reactions to Kayseri, Eskişehir and Ankara isolates, respectively. Landraces #9 and #21 showed resistant responses to two isolates and showed intermediate response to one isolate. Landraces #3 and #22 showed resistant reactions to one isolate and showed intermediate reactions to two isolates. Landraces #7 and #11 showed intermediate responses to all isolates. Landraces that showed resistant and/or intermediate reactions could be integrated into barley breeding programs.

Key words: *Barley stripe, Drechslera graminea, Pyrenophora graminea, barley landraces, disease resistance*

INTRODUCTION

Barley (*Hordeum vulgare* L.) is an important crop in world agriculture. There are numerous diseases affecting barley yield and quality (Mathre, 1982). Barley stripe disease is a seed transmitted disease and it is caused by the fungus *Drechslera graminea* (Rab.) Shoem. (teleomorph: *Pyrenophora graminea* S. Ito & Kuribay.). Barley stripe is an important disease of barley in Iran and Turkey (Golzar, 1993; Zad *et al*., 2002: Karakaya *et al*., 2016).Ten to 15% yield losses due to this disease have been reported in Turkey (Aktaş, 1984). Karakaya *et al*. (2016) reported barley stripe disease in 40% of the fields inspected in Central Anatolia region of Turkey. In

---

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Ankara Univ., Fac. of Agricult., Dept. of Plant Protect., Ankara, Turkey
[2] Urmia Univ., Fac. of Agriculture, Dept. of Agronomy, Urmia, West Azerbaijan, Iran Correspondence: Aziz Karakaya, Ankara Univ. , Fac. of Agricult., Dept. of Plant Protect., Ankara, Turkey
E-mail: karakaya@agri.ankara.edu.tr

these fields, disease incidence ranged between 1%-70%. This disease is especially important in areas that no seed treatment is practised or diseased seeds are used for seeding. Barley landraces have good adaptation capabilities to environmental conditions. They have tolerance to biotic and abiotic stress agents. *Hordeum vulgare* ssp. *spontaneum*, the progenitor of cultivated barley, is common in Fertile Crescent region. Movement of genes from wild barley to landraces may occur. Because of these characteristics, barley landraces are important genetic resources (Allard and Bradshaw 1964; Newton *et al*., 2010; Ceccarelli and Grando, 1999). Barley landraces are commonly grown in Fertile Crescent region and they are still planted in Iran and Turkey. In this study, barley landraces obtained from 4 different regions of northwest Iran were evaluated for their resistance status to 3 *Drechslera graminea* isolates in order to find sources of resistance to barley stripe disease.

MATERIALS AND METHODS

Plant materials

Barley landraces were obtained from Oshnaviye, Piranshahr, Bukan and Naghadeh regions of northwest Iran. Barley landrace samples were taken from the farmers' fields by random sampling. Healthy looking individual seeds from different regions were selected. Black colored seeds were also included. One of the landraces was 6-rowed (landrace no: 9) and the remaining landraces were 2-rowed. Kernels of the five landraces were black (landrace numbers 3, 5, 7, 16 and 24) and kernel color of the remaining landraces were white. Individiual seeds were planted in 18 cm in diameter plastic pots filled with field soil. These pots were placed to greenhouse bench and seeds were harvested following maturity of landraces. These seeds were used in this experiment.

Isolation

Diseased barley plants were obtained from Kayseri, Eskişehir and Ankara provinces of Turkey where significant amount of barley production occur. Randomly selected 9, 7 and 7 barley fields were inspected in Kayseri, Eskişehir and Ankara provinces, respectively. From these fields, 4, 4 and 2 diseased plants were obtained. Leaf samples were surface-sterilized with 1% NaOCl solution for 1 minute followed by rinsing with sterile water and then incubated in Petri plates containing sterile moist filter paper until sporulation. After sporulation of the fungus, single spores were taken under a stereomicroscope and transferred to Potato Dextrose Agar (PDA) plates. Three randomly selected isolates from Kayseri, Eskişehir and Ankara provinces were used in this experiment.

Preparation of inoculum and inoculation

Sandwich method outlined by Mohammad and Mahmood (1974) was used. Barley seeds were surface sterilized with 1% NaOCl solution for 3 minutes followed by rinsing with sterile water. Ten seeds were placed onto 10 days old *Drechslera gramineae* cultures grown in PDA and a sandwich was formed. There were three replications. Petri plates with seeds were maintained 72 hours at 22°C and they were further incubated 5-7 days at 4 °C. After this period, seeds were placed into pots, 16 cm in diameter, containing sand, animal manure, and soil (w:w 1:1:3). Plants were grown in a greenhouse with a night/day temperature of 10–22 ± 3°C (Çelik *et al*., 2016).

Disease assesment

Plants were evaluated 60 days later using a scale developed by Tekauz (1983). In this scale, plants were classified according to infection percentages of genotypes. Infection percentage was defined as the number of infected genotypes / total number of genotypes × 100). If percent plant infection was less than 5% then that genotype was considered as resistant (R). Plant infection percentages between 5%-17% considered as intermediate (I) reaction. If more than 17% of the plants diseased then that genotype considered as susceptible (S). Virulence differences among the isolates were subjected to analysis of variance and means were separated using LSD test (MSTAT, Michigan State University, East Lansing, Michigan, USA).

RESULTS AND DISCUSSION

Barley landraces exhibited different reactions to *Drechslera graminea* isolates (Table 1). Also, isolates differed for their pathogenicity to landraces. One, five and six landraces showed resistant reactions to Kayseri, Eskişehir and Ankara isolates, respectively. Six, eleven and eleven landraces showed intermediate reactions to Kayseri, Eskişehir and Ankara isolates, respectively. Landraces #9 and #21 showed resistant responses to two isolates (no disease was observed in plants) and showed intermediate response to one isolate (6.6% of the plants diseased). Landrace #9 was a 6-rowed landrace. Landraces #3 and #22 showed resistant reactions to one isolate and showed intermediate reactions to two isolates. Landraces #7 and #11 showed intermediate responses to all isolates. Black colored landraces exhibited resistant and/or intermediate reactions to at least one *D. graminea* isolate. Virulence differences among the isolates were observed. Kayseri isolate was significantly more virulent than Ankara and Eskişehir isolates ($P<0.01$). In other studies, virulence differences among the *Drechslera graminea* isolates were also reported (Ulus and Karakaya, 2007; Bayraktar and Akan, 2012; Çelik *et al*., 2016).

In Iran, Golzar (1993) evaluated 50 barley cultivars and advanced lines for resistance to the *Drechslera graminea*. The results of field and greenhouse studies showed that the lines and cultivars Cm67/Apro//Sv.02, As46/Aths•, 109/Mari, a local barley, Deir Alia, 2-2AP, 106/Mzo/DI7I, Kataga/Arabi/Arabi2, Morocco-9-75, Wi 2269 and Moroc-9-75/Pmb were immune to the disease and Roho/Mazurka, Rihane"S", C.63, C108887/C105761, Lignee 527/Arar, C.C.89 and Gloria"S"/Copal"S"-ly-4m-oy were most susceptible to barley stripe fungus. The reactions of other lines and cultivars were between these two categories.

Khodayari *et al*. (2012) investigated the genetic diversity among the Iranian barley landraces using 17 microsatellite markers. They found a high level of genetic diversity between the barley landraces in Iran. The authors concluded that Iranian barley gene pool was a valuable source to search for new useful alleles for crop improvement. Also Ebrahimi *et al*. (2013) reported high level of genetic diversity in wild and landraces of barley which could be used for improvement of barley cultivars.

Table 1. Response of 25 Iranian barley landraces to 3 isolates of *Drechslera graminea* 60 days after inoculation. For evaluation, a 1-3 scale developed by Tekauz (1983) was used (R: Resistant, I: Intermediate; S: Susceptible).

Landrace No	Kayseri isolate		Eskişehir isolate		Ankara isolate	
	Mean disease percent	Scale value	Mean disease percent	Scale value	Mean disease percent	Scale value
1	83.3	3 (S)	50	3 (S)	23	3 (S)
2	13.3	2 (I)	17.6	3 (S)	18.2	3 (S)
3	14.2	2 (I)	0	1 (R)	16.6	2 (I)
4	33.3	3 (S)	15.7	2 (I)	18.2	3 (S)
5	23	3 (S)	20	3 (S)	0	1 (R)
6	75	3 (S)	26.3	3 (S)	4.7	1 (R)
7	10	2 (I)	10	2 (I)	10	2 (I)
8	50	3 (S)	30	3 (S)	40	3 (S)
9	0	1 (R)	0	1 (R)	6.6	2 (I)
10	25	3 (S)	11.1	2 (I)	55.5	3 (S)
11	5.5	2 (I)	10	2 (I)	5.5	2 (I)
12	53.8	3 (S)	22.2	3 (S)	11.7	2 (I)
13	38.4	3 (S)	20	3 (S)	41.6	3 (S)
14	57.1	3 (S)	36.4	3 (S)	30	3 (S)
15	22.2	3 (S)	11.7	2 (I)	0	1 (R)
16	18.8	3 (S)	13.3	2 (I)	7.6	2 (I)
17	17.6	3 (S)	11.1	2 (I)	6.3	2 (I)
18	27.7	3 (S)	10.5	2 (I)	0	1 (R)
19	37.5	3 (S)	21.4	3 (S)	0	1 (R)
20	44.4	3 (S)	7.6	2 (I)	66.6	3 (S)
21	12.5	2 (I)	4.7	1 (R)	0	1 (R)
22	5.8	2 (I)	4.7	1 (R)	5.8	2 (I)
23	20	3 (S)	6.6	2 (I)	8.3	2 (I)
24	23	3 (S)	0	1 (R)	10	2 (I)
25	66.6	3 (S)	7.6	2 (I)	12.5	2 (I)
Mean*	31.12±4.49a		14.74±2.37b		15.95±3.57b	

*Means not connected by same letter are significantly different (LSD: 13.44) (P<0.01).

Barley landraces are good sources for obtaining disease resistant barley cultivars. Abdel-Ghani *et al*. (2008) screened barley landraces collected from diverse ecogeographical regions of Jordan for resistance to powdery mildew. They found that most barley landraces of all tested lines were highly resistant to powdery mildew disease. Çelik Oğuz *et al*. (2017) found resistance among the Turkish barley landraces to both forms of net blotch. In the current study, we identified Iranian barley landraces showing resistant and/or intermediate responses to *Drechslera graminea* isolates. These landraces could be used for improvement of barley.

CONCLUSIONS

Large variation was found among the barley landraces obtained from northwest Iran to 3 isolates of *Drechslera graminea*. Resistance was found in Iranian barley landraces to barley stripe fungus. Barley landraces that showed resistant and/or intermediate reactions to *D. graminea* isolates could be used in resistance breeding studies.

REFERENCES

Abdel-Ghani, A. H., Al-Ameiri, N. S., Karajeh, M. R. (2008): Resistance of barley landraces and wild barley populations to powdery mildew in Jordan. Phytopathologia Mediterranea, 47: 92–97.

Aktaş, H. (1984): Spread of leaf spots in barley growing areas of Turkey. Proc. 6th Congr. Un. Phytopth. Mediterr. Cairo, Egypt. 338-341.

Bayraktar, H., Akan, K. (2012): Genetic characterization of *Pyrenophora graminea* isolates and the reactions of some barley cultivars to leaf stripe disease under greenhouse conditions. Turkish Journal of Agriculture and Forestry 36: 329-339.

Ceccarelli, S., Grando. S. (1999): Barley landraces from the Fertile Crescent. a lesson for plant breeders. Pages 51-76 In: Genes in the field-on-farm conservation of plant diversity (S. B. Brush, ed.). International Plant Genetic Resources Institute, International Development Research Centre, Lewis Publishers, Boca Raton, London, New York, Washington DC.

Çelik Oğuz, A., Karakaya, A., Ergün, N., Sayim İ. (2017): Turkish barley landraces resistant to net and spot forms of *Pyrenophora teres*. Phytopathologia Mediterranea, 56(2): 217-223.

Çelik, Y., Karakaya, A., Oğuz, A. Ç., Mert, Z., Akan, K., Ergün, N., Sayim, İ. (2016): Determination of the reactions of some barley landraces and cultivars to *Drechslera graminea*. Akdeniz Üniversitesi Ziraat Fakültesi Dergisi, 29(2): 43-47.

Ebrahimi, A., Naghavi, M. R., Sabokdast, M., Sarabshelli, A. M., Ghaderdan., K. (2013): Evaluation of genetic diversity of Iranian wild barley (*Hordeum* sp.) and landraces using morphological characters. Iranian Journal of Rangelands and Forests Plant Breeding and Genetic Research, 21(1) pp.Pe56-Pe66, En67.

Golzar H. (1993): Resistance evaluation of cultivars and advanced lines to barley leaf stripe disease caused by *Pyrenophora graminea* in northern Iran. In: Jacobs

T., Parlevliet J.E. (eds.) Durability of Disease Resistance. Current Plant Science and Biotechnology in Agriculture, vol 18. Springer, Dordrecht.

Karakaya, A., Mert, Z., Çelik Oğuz, A., Çetin, L. (2016): Distribution of barley stripe disease in Central Anatolia, Turkey. Selcuk Journal of Agriculture and Food Sciences, 30(2): 58-61.

Khodayari, H., Saeidi, H., Roofigar, A. A., Rahiminejad, M. R., Pourkheirandish, M., Komatsuda, T. (2012): Genetic diversity of cultivated barley landraces in Iran measured using microsatellites International Journal of Bioscience, Biochemistry and Bioinformatics, 2(4): 287-290.

Mohammad, A., Mahmood, M. (1974): Inoculation techniques in Helminthosporium stripe of barley. Plant Disease Reporter, 58:32-34.

Tekauz, A. (1983): Reaction of Canadian barley cultivars to *Pyrenophora graminea*, the incitant of leaf barley stripe. Canadian Journal of Plant Pathology, 5: 294-301.

Ulus, C., Karakaya, A. (2007): Assessment of the seedling reactions of some Turkish barley cultivars to barley stripe. Tarım Bilimleri Dergisi, 13 (4): 409-412.

Zad, J., Aghakhani, M., Etebarian, R., Okhovat, M. (2002): Barley leaf stripe disease. Mededelingen - Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, Universiteit Gent 67(2): 279-281.

THE REACTION OF SOME TURKISH BREAD AND DURUM WHEAT CULTIVARS AGAINST TO *Zymoseptoria tritici* (Desm. Quaedvlieg & Crous)*

E. Burcu Turgay[1], Orhan Büyük[1], Fatih Ölmez[2], Kadir Akan[3],
A. Faik Yıldırım[1]

Original scientific paper

Summary

Septoria Leaf Blight (SLB) caused by *Zymoseptoria tritici* (Desm. Quaedvlieg & Crous) is one of the most crucial wheat leaf diseases in Turkey. Although licensed pest control agents are commonly used against SLB, breeding resistant wheat cultivars can be evaluated as a better approach in terms of more sustainable controlling of the disease. To investigate this potential, 22 bread (*Triticum avestium* L.) and 5 durum wheat (*Triticum turdigum* L.subsp. *durum*) cultivars commonly cultivated in Central Anatolia were used in the experiment from 2015-2016 in Ankara District, Turkey. In this research, E-17 and A-69 *Zymoseptoria tritici* isolates with a different virulence have been used to experimentally infect selected cultivars in a field experiment, designed as randomized block design with 4 replicates. The results showed that the disease severity of the cultivars was significantly different and changed depending on the isolates (cultivar x isolate interactions) (F = 5,738; p = 0.00; CV = 16.76). Additionally, our results indicated that the most tolerant bread wheat cultivars to *Zymoseptoria tritici* were Tosunbey, Ekiz, Yüreğir-89, Pehlivan and Bezostaja-1 and the most susceptible durum wheat cultivar was Çeşit 1252.

Key words: *Septoria leaf blight, Zymoseptoria tritici, wheat, disease resistance*

INTRODUCTION

Septoria Leaf Bloch (SLB) caused by *Zymoseptoria tritici* (formerly known as *Mycosphaerella graminicola*) is currently one of the most important fungal foliar diseases of wheat in many regions of the world (Cowger *et al.*, 2000). SLB was found to reduce the yield and the quality of wheat also in Turkey (Finci, 1982). Yield losses attributed to heavy incidences of SLB of wheat have been observed to range from 31 to 53% and the kernel of vulnerable wheat cultivars have been reported to shrivel and not fit for milling (Eyal, 1981, King *et. al.*, 1983, Eyal *et. al.*, 1987).

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Plant Protection Central Research Institute Yenimahalle, Ankara, Turkey
[2] Central Research Inst. for Field Crops, Yenimahalle, Ankara, Turkey
[3] Ahi Evran Univ., Fac. of Agricult., Dept. of Plant Protect., Kırşehir, Turkey Correspondence: E. Burcu Turgay, Plant Protection Central Research Institute Yenimahalle, Ankara, Turkey
Email:cercospora79@gmail.com

Several fungicides are currently used to control SLB. Usage of fungicides is usually costly and causes resistance to some groups of chemicals in fungus population in general. Recently, isolates of *Z. tritici* were commonly found to be resistant to strobilurin fungicides (Qo inhibitor, QoI) in Europe and there has been a wide failure of QoI fungucides to control SLB (Anonymous, 2003). Therefore, using fungicides to control SLB seems not adequate as a sole control strategy. Breeding and growing cultivars resistant to SLB can provide an effective, economic and environmentally-safe strategy to reduce yield losses. Such crops are normally associated with the maximum yield potential. The selection of resistant cultivars appears to be the most feasible method for controlling this disease. Although there are several studies regarding the development of resistant cultivars to this diseases in different countries (Dubin and Rajaram, 1996; Eyal, 1999; Cowger *et al*. 2000), there were only two studies about the field resistance status of the wheat cultivars grown in Thrace and Mediterranean region of Turkey (Finci, 1982, Canıhoş *et.al*., 1997). Finci (1982) found an infection ranging between 28 to 40% in approximately 20% of the fields surveyed.

Majority of the studies about plant disease indicated that using resistant cultivars was the most economical way of controlling the disease (Arraiano and Brown, 2006). Determination of the resistance source before designing a breeding program for any disease is a big challenge because it needs to screen a set of cultivars against the disease population composed of different virulence levels of isolates. The pathogenic variability of SLB has not been studied previously in Turkey and the status of recently registered wheat cultivars to the disease is unknown. No research has been conducted in relation to SLB since Finci (1982) and Canıhoş (1997). Therefore, our knowledge about SLB is very limited in Turkey. The objective of the present study was to determine the resistance status of some registered Turkish bread and durum wheat cultivars grown in Central Anatolia which is the most important wheat producing area of Turkey.

MATERIAL AND METHODS

Wheat cultivars

Twenty two bread (*Triticum avestium* L.) and 5 durum (*Triticum turdigum* L.subsp. *durum*) wheat cultivars (Table 4) bred by the Central Research Institute for Field Crops (CRIFC) in Turkey were used in the study.

Fungal Isolates

Isolates E-17 and A-69 of *Z. tritici* were obtained from field survey in Ankara, Konya, Eskişehir and Kırşehir provinces of Central Anatolia which is the most important wheat growing areas in Turkey during 2013 and 2014 (Turgay *et al*. 2016). Pathology tests were carried out on adult plant technique with slight modification (Eyal and Scharen, 1977). The symptoms of the disease were observed after 10 days and picnidios after 15 days. Final plant evaluation was performed within 21 day after the inoculation, when the reaction of the susceptible control was a maximum. The reactions were evaluated by using a 0-5 rating scale developed by Rosielle (1972) and slightly modified by Mc Cartney *et al*., (2002). Values 0 to 2 were considered as resistance (Avirulent) and values 3 to 5 were considered as susceptibility (virulent) (Table1).

Table 1. Rating scale (0-5) used in evaluation of Septoria disease

Scale value	**Plant symptoms**
0- Immune (Imm)*	No pycnidial formation, no symtoms or occasional hypersensitive fleck
1-Highly Resistance (HR)	No or only occasional isolated pycnidia formed, particulary in older leaf tissue, hypersensitive flecking in younger leaf tissue
2-Resistance (R)	Very light pycnidial formation. Some coalescing of lesions mainly toward the leaf tip and in older leaf tissue
3-Intermediate (MR)	Light pycnidial formation. Coalescing of lesions normally noticeable towards the leaf tip and elsewhere on the leaf
4-Susceptible (S)	Moderate pycnidial formation, lesions coalescing considerably
5-Very Susceptible (VS)	Large abundant pycnidia, lesions coalescing extensively

*Imm (Immune):0, Highly Resistance (HR): 5-10%: Resistance (R): 11-19%, Intermediate (MR): 20-29%, Susceptible (S): 30-50%, Very Susceptible (VS): 51% and over

Two *Z. tritici* isolates were used in this study. The isolateA-69 was avirulent on 12 wheat genotypes with different resistance genes to Slb (Slb1-Slb12) (Table-2) and also virulent on Touching-20 which is not resistant to *Z. tritici*. Isolate E-17 was virulent 4 genotypes (Slb-1, Slb-3, Slb-5 and Slb-7) of 12 wheat genotypes with different resistance genes to Slb (Slb-1 - Slb-12) and it was virulent on Touching-20.

Table 2. Differential set of wheat genotypes tested with *Septoria tritici* isolates

Gene	**Chromosome**	**Wheat cultivar/line**	**Reference**
Slb-1	5BL	Bulgaria88	Adhikari *et al*., 2004a
Slb-2	3BS	Veranopolis	Adhikari, 2004b
Slb-3	7AS	Israel 493	Wilson, 1985, resistant to IPO323 (Chartrain *et al*., 2004)
Slb-4	7DS	Tadinia	Adhikari, 2004c
Slb-5	7DS	CS/Synthetic 7D	Arraiano, 2001
Slb-6	3AS	Flame	Branding, 2002
Slb-7	4AL	ST6=Estanzuela Federal	McCartney, 2003

Slb-8	7BL	Synthetic hexaploid W7984	Adhikari, 2003
Slb-9	2BL	Courtot	Chartrain, 2009
Slb-10	1D	Kavkaz-K4500	Chartrain, 2005a
Slb-11	1BS	TE9111	Chartrain, 2005b
Slb-12	4AL	Kavkaz-K4500	Chartrain, 2005a

Field experiment

The field trials were conducted at two different locations, namely Haymana and Yenimahalle of the Experimental Research Station, CRIFC in 2015 and 2016 and two isolates (E-17 and A-69) with a different degrees of virulence. A total of 27 registered Turkish (22 bread and 5 durum) wheat cultivars were evaluated in the experiment. Each trial was established in randomized complete blocks (RCB) with four replicates. Plots of 1.0 x 1.0 m were manually sown with 2 g seed of each cultivar in each line. The distance between the lines were 30 cm.

Both isolates were grown on Malt Yeast Agar (malt extract 4.0 g, yeast extract 4.0 g, sucrose 4.0 g, agar 18.0 g) for 5-7 days before being used for field inoculation. The inoculum concentration was adjusted to 10^7 spores. ml-1. A few drops of Tween 20 (polyoxyethylene–sorbitan monolaurate; Sigma– Aldrich) were added to the inoculum prior to inoculation.
Plants were inoculated with *Z. tritici* isolates at the beginning of tillering (growth stage - GS 21) and the experiment was finalized at Ear emergence from boot (growth stage 51) according to Zadoks *et al*., (1974). Inoculations were repeated four times during this time period. The inoculum was applied in the evening using ultra low volume applicator (ULVA). Following inoculation, plants were covered with transparent polythene material for 24 h to increase humidity and promote infection.

Disease assessment

Diseases severity was assessed as percent of canopy infection when kernels were watery-ripe (GS 70-73). The reactions were evaluated by using a 0-9 Saari-Prescott scale (Saari and Prescott, 1975) (Table-3). Values 0 to 3 were considered as resistance, value 5 was consider as tolerant, and values 7 to 9 were considered as susceptible.

Table 3. Saari-Prescott scale (0-9) used in the evaluation of Septoria disease in field experiment

Scale value	Plant symptoms
0 (Imm)*	No pycnidial formation, no symtoms
1HighlyResistance (HR)	Leaf area has less than 10% necrotic area and no pycnidia
3 Resisitance (R)	Leaf with 11-25% necrotic area and a few pycnidia
5 Intermediate (MR)	Leaf with 26-45% necrotic area and light pycnidial formation
7 Susceptible (S)	Leaf with 46-75% necrotic area and moderate pycnidial formation
9 Very Susceptible (VS)	Leaf with 46-75% necrotic area and large abundant pycnidia

The data obtained were calculated using Towsend-Heuberger equation as given below;

$$(\%) = \Sigma \text{ (nx V / Z x N) x } 100$$

Where n: the number of plants in different scores in the scale
V: scale value
Z: highest scale value
N: Total number of plants is observed
The interactions between isolates and cultivars were evaluated by using multiple comparison test (Duncan's test).

RESULTS AND DISCUSSION

Reaction of 27 cultivars on 2 isolates with different virulence were conducted with artificial inoculation in the field. Variance analysis showed that the disease severity of the cultivars was changed according to the isolates with different virulence values (F = 5,738; p = 0.00; CV = 16,76) (Table 4). Reactions of 22 bread wheat cultivars tested with high virulent E-17 isolate showed that Tosunbey, Es 26, Konya 2002, Ekiz, Aldane, Pehlivan, Kate A-1, Bezostaja-1 and Yüreğir-89 were the most susceptible cultivars to the disease (Table 4). Moreover, reaction of all cultivars tested with low virulent A-69 isolate indicated that Tosunbey, Ekiz, Aldane, Pehlivan, Bezostaja-1 and Yüreğir-89 bread wheat cultivars were the most susceptible cultivars to the diseases.
Although there was a significant statistical interaction between the cultivars and the isolates, Tosunbey, Ekiz, Aldane, Bezostaja-1, Yüreğir-89, and Pehlivan bread wheat cultivars were identified as the most susceptible cultivars to both isolates. While Es 26 bread wheat cultivar was detected to be very sensitive to isolate E-17, the reaction of Es 26 cultivar to the isolate A-69 was found to be moderately resistant. In addition, Konya 2002 bread wheat cultivar was very sensitive to the isolate E-17, but resistant to isolate A-69. Gün-91 and Gerek 79 bread wheat cultivars were found to be tolerant cultivars to both isolates. Bayraktar 2000 and Demir 2000 of bread cultivar were found to be resistant to both isolates.
While Eminbey and Kızıltan-91 cultivars were found to be tolerant to isolate E-17, the same two cultivars were found to be resistant to A-69 isolate. For both isolates, Çeşit 1252 durum wheat cultivar was found to be the most sensitive cultivar to the disease, followed by cultivars of durum wheat Mirzabey 2000 and Kunduru 1149, respectively.

Table 4. Twenty two cultivars of bread and 5 durum wheat cultivars were subjected to analysis of variance and their response to two isolates with different virulence

Disease Severity Mean±Std.error (min-max)		
Bread wheat cultivar	**E-17**	**A-69**
****Gün 91**	32.30±8.42 hl A (10.80-100.00)	42.60±5.4 efghı[1] A[2] (33.20-52.00)
İkizce 96	52.00±0.00efgh B (52.00-52.00)	22.00±6.46 ıjkl A (10.8-33.20)
*****Bayraktar 2000**	22.00±6.46 l A (10.80-52.00)	29.60±10.81 hıjl A (0.00-52.00)
*****Demir 2000**	22.00±6.46 l A (10.80-33.20)	29.60±10.81 hıjl A (0.00-52.00)
Zencirci 2002	70.00±11.48 cdeB (52.00-33.20)	37.90±47.00 fghıj A (33.20-52.00)
***Tosunbey**	100.00±0.00 a B (100.00-100.00)	64.00±69.28 bcdefg A (52.00-76.00)
Kenanbey	42.60±5.42 fghl B (33.20-100.00)	13.70±6.98 jkl A (0.00-33.20)
Lütfibey	58.00±6.00 efgh A (52.00-52.00)	47.30±4.70 efghı A (33.20-52.00)
****Gerek79**	33.20±0.00 hl A (33.20-76.00)	32.30±8.42ghıjk A (10.80-52.00)
Sultan95	37.90±4.70 ghl B (33.20-33.20)	10.80±0.00 jkl A (10.08-10.80)
Altay2000	64.00±6.92 efg A (52.00-52.00)	58.00±6.00 cdefgh A (52.00-76.00)
Sönmez 2001	88.00±6.92abc B (76.00-76.00)	27.60±56.00 hıjk A (10.80-33.20)
Es26	100.00±0.00 a B (100.00-100.00)	37.90±4.70 fghıj A (33.20-52.00)
Karahan 99	88.00±6.92 abc B (76.00-100.00)	37.90±4.70 fghıj A (33.20-52.00)
Konya 2002	100.00±0.00 a B (100.00-100.00)	8.10±2.70 kl A (0.00-10.80)
***Ekiz**	100.00±0,00 a B (100.00-100.00)	76.00±9.79 abcd A (52.00-100.00)
***Kate A-1**	94.00±6,00 ab B (76.00-100.00)	58.00±6,00 cdefgh A (52.00-76.00)
***Pehlivan**	94.00±6,00 ab B (76.00-100.00)	70.00±11.48 bcde A (52.00-100.00)
***Aldane**	100.00±0,00 a A (100.00-100.00)	94.00±6,00 a A (76,00-100,00)
***Bezostaja-1**	76,00±0,00 cde A (76.00-76.00)	82.00±6,00 abc A (76.00-100.00)
***Yüreğir 89**	100.00±0,00 a B (100.00-100.00)	82.00±11,48 ab A (52.00-100.00)
Esperia	70.00±6.00 def A (52.00-76.00)	70.00±11.48 bcde A (52.00-100.00)
Durum wheat cultivar	**E-17**	**A-69**
Kızıltan 91	33.20±0,00 hl B (33.20-33.20)	5.40±3.11 l A (0.00-10.8)
***Çeşit 1252**	82.00±11.48 bcd B (52.00-100.00)	70.00±6.00 bcdef A (52.00-76.00)
Mirzabey 2000	70.00±11.48 cde A	58.00±6.00 cdefghA

	(52.00-100.00)	(52.00-76.00)
Eminbey	32.30±8.42hl B (10.8-52.00)	22.00±6.46 ıjklA (10.80-33.20)
Kunduru 1149	58.00±6.00 efgh B (52.00-76.00)	52.00±0.00 defghıA (52.00-52.00)

***: susceptibile, **: tolerant, ***: resitant**

CONCLUSION

To determine the cultivar resistance and/or tolerance to the disease, highly virulent isolates should be used in cultivar reaction studies. In the present study, the resistance/tolerance status of the 27 wheat cultivars grown widely in the Central Anatolia region was studied.

According to current situation, some tolerant cultivars can be used in the areas where the disease is frequently observed. Selection of these cultivars may decrease the usage of the pecticides and yield loses because of the disease pressure.

In addition, results obtained from current study and finding out resistant and tolerant cultivars will help breeders to develop new cultivars to be used against to Septoria leaf bloch in the future.

ACKNOWLEDGEMENTS

This research was supported by General Directorate of Agricultural Research and Policies (TAGEM).

REFERENCES

Adhikari, T. B., Anderson, J. M., Goodwin, S. B. (2003): Identification and molecular mapping of a gene in wheat conferring resistance to Mycosphaerella graminicola. Phytopathology, 93:1158–1164.

Adhikari, T. B., Yang, X., Cavaletto, J. R., Hu, X., Buechley, G., Ohm, H. W., Shaner, G. (2004a): Molecular mapping of Stb1, a potentially durable gene for resistance to septoria tritici blotch in wheat. Theor Appl Genet 109:944–953.

Adhikari, T. B., Wallwork, H., Goodwin, S. B. (2004b): Microsatellite markers linked to the Stb2 and Stb3 genes for resistance to septoria tritici blotch in wheat. Crop Sci., 44:1403–1411.

Adhikari, T. B., Cavaletto, J. R., Dubcovsky, J., Gieco, J. O., Schlatter, A. R., Goodwin, S. B. (2004c): Molecular mapping of the Stb4 gene for resistance to septoria tritici blotch in wheat. Phytopathology, 94:1198–1206

Anonymous. (2003): Strobilurin Resistance in *Septoria tritici* in the UK. HGCA Topic Sheet No.69. London, UK: Home Grown Cereals Authority.

Arraiano, L. S., Worland, A. J., Ellerbrook, C., Brown, J. K. M. (2001): Chromosomal location of a gene for resistance to Septoria tritici blotch (Mycosphaerella graminicola) in the hexaploid wheat Synthetic 6x. Theor Appl Genet, 103:758–764.

Arraiano, L. S. and Brown, K. J. (2006): Identification of isolate-specific and partial resistance to *Septoria tritici* blotch in 238 European wheat cultivar and breeding lines. Plant Pathology 55, 726-738.

Brading, P. A., Verstappen, E. C. P., Kema, G. H. J., Brown, J. K. M. (2002): A gene-for-gene relationship between wheat and Mycosphaerella graminicola, the Septoria tritici blotch. Phytopathology 92:439– 445.

Chartrain, L., Brading, P. A., Makepeace, J. C., Brown, J. K. M. (2004): Sources of resistance to *Septoria trit*ici blotch and implications for wheat breeding. Plant Pathol., 53:454–460.

Chartrain, L., Berry, S. T., Brown, J. K. M. (2005a): Resistance of wheat line Kavkaz-K4500 L.6.A.4 to *Septoria tritici* blotch controlled by isolate-specific resistance genes. Phytopathology, 95:664–671.

Chartrain, L., Joaquim, P., Berry, S. T, Arraiano, L. S., Azanza, F., Brown, J. K. M. (2005b): Genetics of resistance to septoria tritici blotch in the Portuguese wheat breeding line TE 9111. Theor Appl Genet., 110:1138–1144.

Chartrain, L., Brading, P. A., Brown, J. K. M. (2005c): Presence of Stb6 gene for resistance to septoria tritici blotch (Mycosphaerella graminicola) in cultivars used in wheat-breeding programmes worldwide. Plant Pathol., 54:134–143.

Chartrain, L., Sourdille, P., Bernard, M., Brown, J. K. M. (2009): Identification and location of Stb9, a gene for resistance to septoria tritici blotch in wheat cultivars Courtot and Tonic. Plant Pathology, 58: 547–555.

Canıhoş, Y., Yağbasanlar T., Kurt, Ş., Toklu, F. (1997): Çukurova Bölgesi'nde bazı önemli buğday çeşit ve hatlarının sarı pas ve septorya yaprak lekesi hastalıklarına karşı reaksiyonları. Ç.Ü.Z.F. Dergisi, 1997, 12, (3):89-98.

Cowger, C., Hoffer, ME., Mundt, CC. (2000): Specific adaptation by *Mycosphaerella graminicola* to a resistant wheat cultivar. Plant Pathology, 49, 445–51.

Dubin, HJ., Rajaram, S. (1996): Breeding disease-resistant wheats for tropical highlands and lowlands. Annual Review of Phytopathology, 34, 503–26.

Eyal, Z., Scharen, A. L. (1977): A quantitative method for the inoculation of wheat seedlings with pycnidiospores of *Septoria nodorum*. Phytopathology, 67:712-714.

Eyal, Z. (1981): Integrated control of Septoria diseases of wheat. Plant Dis., 65:763-768.

Eyal, Z. Scharen, A. L., Prescott, J. M., Ginkel, M. (1987): The Septoria Diseases of Wheat: Concepts and methods of disease management, Mexico, D. F.: CIMMYT. 11-20.

Eyal, Z. (1999): The *Septoria tritic*i and *Stagonospora nodorum* blotch diseases of wheat, European Journal of Plant Pathology, 105, 629–41.

Finci, S. (1982): Studies on the species, distribution of septoria fungı observed in wheat growing area amd varietal reactions in Turkey. Plant Protection Bulletin, 22 (2) :72-88.

King, J. E., Cook, R. J., Melvılle, S. C. (1983): A review of Septoria diseases of wheat and barley. Annuals of Applied Biology, Volume 103, Issue 2, Pp: 345–373.

McCartney, C. A., Bruˆle´-Babel, A. L., Lamari, L., Somers, D. J. (2003): Chromosomal location of a race-specific resistance gene to Mycosphaerella graminicola in the spring wheat ST6. Theor Appl Genet 107:1181–1186.

Saari, E. E., and Prescott, J. M. (1975): A scale for appraising the foliar intensity of wheat diseases. Plant Dis. Rep., 59:377-379.

Turgay, E. B., Büyük, O., Ölmez, F., Yıldırım, A. F. (2016): Determination of prevalence of the Septoria leaf blotch *Zymoseptoria tritici* (Desm.Quaedvlieg&Crous) in Central Anatolia and its molecular identification. Plant protection Bulletin, 56 (3).

Zadoks, J. C., Changi, T. T., Konzak, C. F. (1974): A decimal codefor the growth stages of cereals. Weed Res., 14, 415–421.

FIRST REPORT OF *CUCUMBER MOSAIC VIRUS* IN GARLIC MUSTARD IN BOSNIA AND HERZEGOVINA*

PRVI NALAZ VIRUSA MOZAIKA KRASTAVCA NA ČEŠNJAČI U BOSNI I HERCEGOVINI

Anesa Jerković-Mujkić[1], Senka Čaušević[1], Azra Ćerimović[1], Renata Bešta-Gajević[1]

Original scientific paper

Rezime

U proljeće 2016. godine na brojnim primjercima češnjače (*Alliaria petiolata* (M. Bieb.) Cavara & Grande) na više lokaliteta u području Sarajeva zapazili smo simptome virusnog oboljenja u vidu lisnog mozaika i kržljavosti. Iste simptome smo primijetili i prethodnih godina na istim lokalitetima.
Simptomatični listovi su korišteni za mehanički prijenos na sljedeće test-biljke: *Cucumis sativus* L. \`Cornichon\`, *Cucurbita pepo* L. \`Greyzini\`, *Capsicum annuum* L.\`Sirvija\`, *Nicotiana rustica* L. i *Phaseolus vulgaris* L. \`Top Crop\`. Prijenos virusnog izolata vršen je iz zaraženih na zdrave test-biljke pomoću zdravih lisnih uši *Myzus persicae* Sulzer and Aphis (Hemiptera: Aphididae). Reakcije inokuliranih test-biljaka ukazivale su na infekciju češnjače virusom mozaika krastavca (CMV). Radi potvrde CMV infekcije, testirane su prirodno inficirane biljke *A. petiolata* kao i mehanički inokulirane test-biljke ELISA metodom uz pomoć komercijalnih detekcijskih kitova.
Rezultati naših istraživanja su potvrdili prisutnost CMV u zaraženim biljkama češnjače. Ovaj nalaz pokazuje da biljke *A. petiolata* mogu služiti kao prirodni rezervoar CMV infekcije, iz kojih se ovaj ekonomski značajan virus može širiti na druge samonikle i kultivirane biljke.

Ključne riječi: *češnjača, virus mozaika krastavca, ELISA, rezervoar*

Summary

Symptomatic leaves were used for mechanical transmission to test plants: *Cucumis sativus* L. \`Cornichon\`, *Cucurbita pepo* L. \`Greyzini\`, *Capsicum annuum* L. \`Sirvija\`, *Nicotiana rustica* L. and *Phaseolus vulgaris* L. \`Top Crop\`. Transmission of virus isolate from infected to healthy test plants was conducted by healthy aphids *Myzus persicae* Sulzer and Aphis (Hemiptera: Aphididae). Reactions of inoculated test plants indicated infection of garlic mustard with cucumber mosaic virus (CMV). Naturally

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Faculty of Science, University of Sarajevo, Bosnia and Herzegovina

infected *A. petiolata* plants and mechanically infected test plants were ELISA-tested to verify CMV infection using commercial detection kits.
Results of our investigation confirmed the presence of CMV in infected garlic mustard. This finding indicate that *A. petiolata* could serve as natural reservoir of CMV infection from which this economically important virus can spread to the other wild and cultivated plants.

Key words: *garlic mustard, cucumber mosaic virus, ELISA, reservoir*

INTRODUCTION

Cucumber mosaic virus (CMV) is one of the *most widely spread plant viruses in nature*. CMV has the widest host range for any plant virus, including more than 1200 plant species in over 100 families (Palukaitis and Garcia-Arenal, 2003). The virus is transmissible in a non-persistent manner by more than 80 species of aphids in 33 genera (Edwardson and Christie, 1991), amonng them *Myzus persicae* and *Aphis gossypii* are two important vectors.

Garlic mustard (*Alliaria petiolata* (M. Bieb.) Cavara and Grande is an herbaceous plant in the family Brassicaceae. Native to Europe, it has naturalized in North America, North Africa, India, Sri Lanka and New Zealand (Tunyalee and Martin, 2000). The plant is classified as an invasive species in North America (Nuzzo, 1993; Nature Conservancy of Canada (NCC), 2007)**.** The common name garlic mustard is derived from the characteristic garlic odor when stems and leaves are crushed. This biennial plant spends the first growing year as a rosette and flowers in the second growing year. Garlic mustard frequently occurs in moist shaded soils of roadsides, forest openings, edges of woods, trail edges and in urban areas.(Mayer *et al*., 2011). Within its native range, garlic mustard is host plant for seven fungi and several viruses including cucumber mosaic virus, turnip mosaic virus and white clover mosaic virus (Polak, 1985; Zhao *et al*., 2016). In Bosnia and Herzegovina CMV has been isolated previously from *Trifolium sp.*, (Grbelja, 1974), *Beta vulgaris* L. (Buturović *et al.*, 1980), *Pisum sativum* L. (Štefanac *et al.*, 1981), *Buddleia davidii* Franch. (Erić and Grbelja, 1985; Jerković-Mujkić *et al.*, 2005), *Ligustrum vulgare* L. (Grbelja *et al.*, 1986; Jerković-Mujkić *et al*., 2012), *Lamium maculatum* L. (Bešta-Gajević *et al*., 2013) and *Chenopodium album* L. (Bešta-Gajević *et al*., 2014).

In spring 2016, many garlic mustard plants with virus-like symptoms consisting of leaf mosaic and stunting were found on several locations in Sarajevo area. The same symptoms are seen on garlic mustard in previous years at these locations. In this study,we present the isolation and identification of viral agent causing disease in *Alliaria petiolata* in Bosnia and Herzegovina.

MATERIAL AND METHODS

Virus source Naturally infected garlic mustard plants were used as a source of virus. Leaves showing disease symptoms were collected in plastic bags kept at 4 ºC and transferred to laboratory.

Mechanical transmission Virus was isolated by mechanical transmission, using inuculum prepared by homogenizing infected plant tissue in 0.1M phosphate buffer pH 7. Crude sap extracted from naturally infected garlic mustard leaf samples was rubbed on test plant leaves dusted with 600 mesh carborundum for virus propagation. For infective assays the following test plants: *Cucumis sativus* L. `Cornichon`, *Cucurbita pepo* L. `Greyzini`, *Capsicum annuum* `Sirvija`, *Nicotiana rustica* L. and *Phaseolus vulgaris* L. `Top Crop`. All experimental hosts were grown in controlled environment room (23 °C and 18 hours of artificial light per day) in the Laboratory for Plant virology, Faculty of Science Sarajevo. Observations were made for symptom development every day.

Transmission by aphids Adult healthy aphids *Myzus persicae* Sulzer and Aphis of the same age (7-9 days old) were used for transmission experiments. Aphids were placed in plastic dishes and subjected to a 1 h pre-acquisition starvation period. After that, aphids were settled on the leaf of an infected source plant for a 5 min acquisition access period. Groups of 5 aphids were placed to test plants for a 2 h-inoculation period. Then, all plants were sprayed with an insecticide and placed in an aphid-free chamber in controlled environmental conditions .

DAS-ELISA test Symptomatic naturally infected and inoculated test plants were analyzed by serological test for the identification of virus isolate. Collected leaf samples were tested for the presence of cucumber mosaic virus (CMV) by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Clark and Adams, 1977). ELISA reagents, positive and negative controle and microplates were supplied by DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany) and used according to the manufacturer's recommendation. Leaf tissue were extracted in extraction buffer (PBS-Tween + 2% PVP) in 1/20 (v/w) ratio. The color development was measured at 405 nm with an ELISA reader (MULTISKAN MCC/340) after 30–120 min. Samples were considered positive when absorbance values reached three times the mean value of the negative healthy control.

RESULTS AND DISCUSSION

A virus disease of garlic mustard (*Alliaria petiolata* (M. Bieb.) Cavara & Grande) was observed showing symptoms of mosaic, mottling, vein banding and deformation of the leaves (Figure 1). Stems of infected plants were small, the internodes short and the plants markedly stunted.

Figure 1. Virus symptoms on the leaf of garlic mustard

The virus was transmitted to the healthy test plants by mechanical inoculation. Five plant species were used to demonstrate infectivity and to identify the virus, and the virus was detected in all of them. Incubation period ranged from 7 to 15 days, depending of test plant species. Results of bioassays were presented in Table 1 and Figure 2. Reactions of the test plants, and particulary that of *Cucumis sativus* and *Nicotiana rustica* were typical of cucumber mosaic virus described in the literature (Brunt *et al.*, 1996; Palukaitis and Garcia-Arenal, 2003).

Table 1. Expression of symptoms on inoculated test plants

No	Test plants	Local	Systemic
1	Cucumis sativus L. \`Cornichon\`	Diffuse yellow spots	Leaf mosaic, variegation and deformation
2	*Cucurbita pepo* L. \`Greyzini\`	Diffuse yellow spots	Leaf mosaic, variegation deformation
3.	*Capsicum anuum* \`Sirvija\`	-	Leaf mosaic, mild blistering, reduction of size
4	*Nicotiana rustica* L.	-	Leaf variegation, deformation, blistering, distorsion and reduction of size
5	*Phaseolus vulgaris* L. \`Top Crop\`	-	Leaf chlorotic spots, mosaic

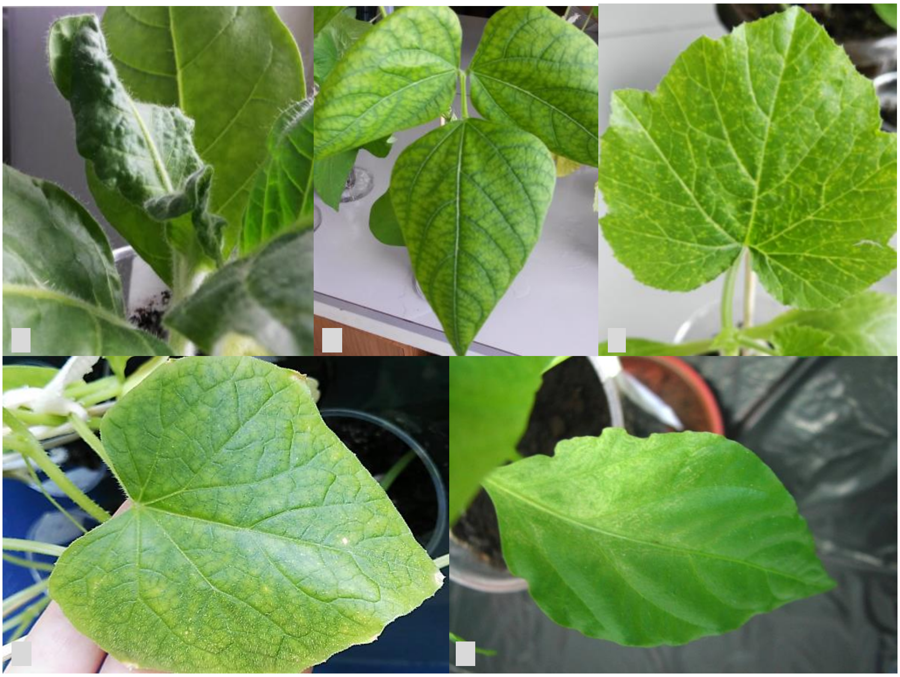


Figure 2. Various symptoms of virus isolate from garlic mustard on test plants: **(a)** *Nicotiana rustica* L.; **(b)** *Phaseolus vulgaris* L.`Top Crop`; **(c)** *Cucurbita pepo* L. `Greyzini`; **(d)** *Cucumis sativus* L. `Cornichon`; **(e)** *Capsicum annuum* `Sirvija`.

Results of experimental transmission by vectors has showed that *Myzus persicae* transmit the virus isolate in non-persistent manner.

Naturally infected garlic mustard and inoculated test plants were assayed by DAS-ELISA and all plants of each species tested positive for cucumber mosaic virus CMV. The results of serological tests were consistent with greenhouse experiments and confirmed that virus isolate from *Alliaria petiolata* belongs to cucumber mosaic virus. According to literature data, this virus is first detected in garlic mustard in former Czechoslovakia (Brčak and Polak, 1963). To our knowledge, our study is the first report of garlic mustard as a natural host of cucumber mosaic virus in Bosnia and Herzegovina. Since CMV can overwinter in biennial and perennial wild plants and weeds, garlic mustard may play certain role in epidemiology of this virus in our region.

CONCLUSION

Based of results of bioassays and ELISA testing it is established that isolate from garlic mustard belongs to cucumber mosaic virus. This finding has expended the list of registered hosts of CMV in Bosnia and Herzegovina. *A. petiolata* could serve as natural reservoir of CMV infection from which this economically important virus can spread to the other wild and cultivated plants.

REFERENCES

Bešta-Gajević, R., Jerković-Mujkić, A., Pilić, S., Stanković, I., Vučurović, A., Bulajić, A., Krstić, B. (2013): *Lamium maculatum* is a Natural Host for Cucumber mosaic virus. Plant Disease, The American Phytopathological Society, 97(1), 150-151.

Bešta-Gajević, R**.**, Jerković-Mujkić, A., Pilić, S. (2014): Coinfection of *Chenopodium album* L. with *Cucucmber mosaic virus* and *Cherry leaf roll virus*. *Proceedings of 24th International Scientific-Expert Conference of Agriculture and Food Industry*, Sarajevo, 413 – 416.

Brcak J., Polak Z. (1963): Identification of the viruses responsible for the mosaic disease of Alliaria officinalis Andr. in central Bohemia. Preslia, 35:110-117

Brunt, A. A, , Crabtree, K., Dallwitz, M. J. et al. (1996): Viruses of Plants. Descriptions and Lists from VIDE Database. Cambridge,

Buturović, D., Grbelja, J., Erić, Ž. (1980): Proučavanje viroza mozaika krastavca na šećernoj repi. *Zaštita bilja* 31(2), 129-135.

Clark, M. F., Adams, A. N. (1977): Characteristics of a microplate method of enzyme-linked immunosorbent assay for the detection of pant viruses. J. Gen. Virol. 34, 475-483.

Edwardson, J. R., Christie, R. G. (1991): Cucumoviruses. In 'CRC handbook of viruses infecting legumes'. CRC Press, Boca Roton. 293–319.

Erić, Ž., Grbelja, J. (1985): Occurrence of cucumber mosaic virus on *Buddleia davidii* in Yugoslavia. *Acta Bot. Croat* 44, 11-14.

Grbelja, J. (1974): Istraživanje virusnih izolata iz djeteline. IV Kongres biologa Jugoslavije Sarajevo, rezime referata 133.

Grbelja, J., Erić, Ž., Jenkić, Z. (1986). Nalaz virusa mozaika krastavca (cucumber mosaic virus) na kalini (*Ligustrum vulgare* L.) u Jugoslaviji. Godišnjak Biol. Inst. Univ. u Sarajevu 39, 33-37.

Jerković-Mujkić, A., Šašić, M., Bešta, R. (2005): Nalaz virusa mozaika krastavca u ukrasnom grmlju na području Sarajeva. XVIII naučno-stručni skup poljoprivrede i prehrambene industrije, Neum.

Jerković-Mujkić, A. , Delić, O., Bešta, R. (2012): Biodiverzitet biljnih virusa urbanog zelenila Sarajeva. Radovi ANUBIH, Knjiga 22, Sarajevo 2012

Mayer, M., Hudson, W., Robbins, G. (2011): Annual Report on Garlic Mustard, Alliaria petiolata, an Alien Invader of NJ’s Deciduous Forests. New Yersey Department of agriculture.

Nature Conservancy of Canada (NCC). (2007): Control Methods for the Invasive PlantGarlic Mustard (Alliaria petiolata) within Ontario Natural Areas.V1.0. NCC – Southwestern Ontario Region, London, Ontario. 16 pp.

Nuzzo, V. A. (1993): Distribution and spread of the invasive biennial garlic mustard (*Alliaria petiolata*) in North America, pp. 137–146. *In* B. N. McKnight [ed.], Biological Pollution. Indiana Academy of Science, Indianapolis, IN.

Palukaitis, P., García-Arenal, F. (2003): *Cucumber mosaic virus.* CMI/AAB Descriptions of plant viruses No 400.

Polak, Z. (1985): Contribution to the knowledge of the role of wild hosts in the incidence of plant viruses. Mededelingen van de Facultaeit Landbouwwetenschappen Rijksuniversiteit Gent. 50:1301-1304. 70.0 70.1

Štefanac, Z., Grbelja, J., Erić, Ž. (1981): Kukumovirus izdvojen iz graška (*Pisum sativum* L.). Acta Bot. Croat. 40, 35-41.

Tunyalee and Martin (2000): Weed Alert! The Nature Conservancy. Wildland Invasive Species Team http://tncweeds.ucdavis.edu/alert/alrtalli.html

Zhao, K., Margaria, P., Rosa, C. (2016): First Report of White clover mosaic virus and Turnip mosaic virus Mixed Infection on Garlic Mustard in Pennsylvania. Plant disease, Vol. 100, N0 4: 866.

ANTIBACTERIAL ACTIVITY OF *Lavandulla officinalis* L. AND *Thymus serpyllum* L. ESSENTIAL OILS *

ANTIBAKTERIJSKO DJELOVANJE ETERIČNIH ULJA *Lavandulla officinalis* L. I *Thymus serpyllum* L.

Renata Bešta-Gajević[1], Neira Uzunović[1], Senka Čaušević[1], Anesa Jerković-Mujkić[1]

Original scientific paper

Rezime

U posljednje vrijeme jako puno pažnje se poklanja eteričnim uljima aromatičnih i ljekovitih biljaka, kao potencijalnim prirodnim komponentama, u borbi protiv infekcija uzrokovanih mikroorganizmima. Dokazano je da eterična ulja posjeduju ljekovita svojstva te se koriste kao spazmolitici, hepatoprotektivi, antivirusni i antikancerogeni preparati.
Antibakterijsko djelovanje komercijalno dostupnih eteričnih ulja dobivenih iz biljaka *Lavandulla officinalis* L. i *Thymus serpyllum* L. (Bioaromatica, Croatia) *in vitro* je testirano difuzionom metodom udubljenja u agaru. U istraživanju su korištena četiri referentna soja bakterija: *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027 i *Bacillus subtilis* ATCC 6633 kao i pet izolata *Klebsiella pneumoniae, Escherichia coli, Shigella flexneri*, *Salmonella enteritidis* i *Proteus mirabilis* dobivenih iz kliničkih uzoraka (KUC Tuzla).
Ispitivana eterična ulja pokazala su značajno baktericidno djelovanje. Naši rezultati ukazuju da eterično ulje majčine dušice ima snažniju antimikrobnu aktivnost u poređenju sa eteričnim uljem lavande. Testirane Gram-negativne bakterije bile su više osjetljive na ispitivana eterična ulja nego Gram-pozitivne bakterije.

Ključne riječi: *antibakterijska aktivnost, difuziona metoda udubljenja u agaru, Lavandulla officinalis L., Thymus serpyllum L.*

Summary

Essential oils from aromatic and medical plants receive particular attention as potential natural agents against microorganisms. Moreover, essential oils are proven to have various pharmacological effects, such as spasmolytic, hepatoprotective, antiviral and anticarcinogenic effects.
Antibacterial activity of essential oils obtained from *Lavandulla officinalis* L. and *Thymus serpyllum* L. (Bioaromatica, Croatia) were determined by agar-well diffusion

---

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Prirodno-matematički fakultet Univerziteta u Sarajevu, Faculty of Science, University of Sarajevo.

method. Four referent strains, *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027 and *Bacillus subtilis* ATCC 6633 as well as five clinical isolates (UKC Tuzla) of *Klebsiella pneumoniae*, *Escherichia coli*, *Shigella flexneri*, *Salmonella enteritidis* and *Proteus mirabilis* were used in tests. Our results showed that essential oils exhibited significant bacteriocidal activity. The data indicated that wild thyme essential oil have a higher antimicrobial effects compared to lavander oil. Tested Gram negative bacteria were more suspectible to investigated essential oils than Gram positive bacteria.

Key words: *antibacterial activity, agar-well diffusion, Lavandulla officinalis L., Thymus serpyllum L.*

INTRODUCTION

Recently there have been an increase of attention in the medicinal plants as potential natural antioxidants, antimicrobial, antiviral and anticancer agents. Nowadays it is very important that food has nutritional and medical values. Many scientific works have been focused on finding natural components with various pharmacological activities for use in food industry, perfumery and pharmaceutics in order to replace synthetic antioxidants (Madhavi and Salunkhe 1995; Botterweck *et al.,* 2000; Erenler *et al.,* 2015). Lately, bacterial infections have been increasingly more frequent, primarily due to the resistance of bacteria to synthetic antibiotics. For that reason, it is necessary to improve a natural and safe way of controlling infections caused by bacterial pathogens. Many natural compounds isolated from plants have demonstated a wide spectrum of biological activities. Essential oils are natural plant products containing complex mixture of components which include terpenes and terpenoids and aromatic and aliphatic constituents. Essential oils from aromatic and medical plants receive particular interest as potential natural agents against microorganisms. Antibacterial activity of essential oils depends on chemical composition of their components and their concentration, type of microorganisms and are mainly related to the structure of their cell wall or the structure of the outer membrane (Wesołowska *et al*., 2015). Therefore, biological potential is the result of interactions between different classes of compounds present in etheric oil, although in some studies the activity of essential oils is closely related to the effects of the main components (Burt, 2004). Moreover, essential oils are proven to have various pharmacological effects, such as spasmolytic, hepatoprotective, antiviral, antifungal, insecticidal and anticarcinogenic properties (Lahlou, 2004; Bowles, 2004; Ćavar *et al.,* 2008). They are widely used in medicine for these purposes. Due to accelerated development of drug resistance to human pathogens and the appearance of undesirable effects of certain antifungal agents, the search for new antimicrobial agents is of great concern today (Phongpaichit *et al*., 2005; Höfling *et al*., 2010). Aromatic plants in phytotherapy have a special place which use is justified to a large extent thanks to the biological functions of secondary metabolites (Božin, 2009). *Lavandula officinalis* L.

(lavander) is an aromatic and medicinal plant in the Lamiaceae family with carminative, antispasmodic, antidepressant, expectorant, anti-rheumatic, relaxant, sedative, antiinflammatory properties (Evandri *et al*., 2005; Adam, 2006; Verma *et al*., 2010). Lavander contains a wide diversity of secondary metabolites from which essential oils are the most appreciated biomolecules. Other compounds with documented bioactivities are coumarins, flavonoids and sterols (Lis - Balchin, 2002; Hassanpouraghdam *et al*., 2011). *Thymus serpyllum* L. (wild thyme) belongs to the family Lamiaceae and has been extensively used in official and traditional medicine (Jarić *et al*., 2015). Antiviral, antibacterial and antifungal activity of *Thymus sp.* has been attributed to significant quantities of their phenolic components such as thymol (Rai and Chikindas, 2011). Therefore, the aim of this work was to investigate antimicrobial properties of the essential oils of *Lavandulla officinalis* L. and *Thymus serpyllum* L.

MATERIAL AND METHODS

In this research commercially available essential oils of *Lavandulla officinalis* L. and *Thymus serpyllum* L. were obtained from the Bioaromatica (Croatia) company. The essential oils were dissolved in dimethylsulfoxide (DSMO) to final concentration of 75%, 50% and 25%. Four referent strains, *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027 and *Bacillus subtilis* ATCC 6633 as well as five clinical isolates (UKC Tuzla) of *Klebsiella pneumoniae, Escherichia coli, Shigella flexneri, Salmonella enteritidis* and *Proteus mirabilis* were used in tests. Bacterial strains were cultured overnight at 37 °C on Mueller Hinton broth and adjusted in sterile saline solution to a final density of 0.5 McFarland standard (1.5×10^8 CFU/mL). Antibacterial activity of essential oils was tested by agar-well diffusion method according to slightly modified National Commitee for Clinical laboratory standards (2001). Each dilution of sample was individually introduced into the well. Controls were set up with equivalent quantities of DMSO. The inoculated Petri dishes were kept at 4ºC for 2h and then incubated at 37 ºC for 24h. After incubation time, the diameters of the inhibition zones were measured in millimeters.

RESULTS AND DISCUSSION

In our study of antimicrobial activity of essential oils obtained from *Lavandulla officinalis* L. and *Thymus serpyllum* L. (Bioaromatica, Croatia), both oils exhibited significant bacteriocidal activity. Lavender essential oil has showed the highest antibacterial effects on *Proteus mirabilis* and *Shigella flexneri* strains and on the other hand, the lowest inhibition zones were recorded on *Salmonella enteriditis* and *Bacillus subtilis* (Table 1). The present results and this significant antimicrobial potential of lavandula essential oil supports the results of other authors who also confirmed inhibitory activity of *Lavandula officinalis* essential oil on different bacterial strains

(Rostami *et al.*, 2012). The biological activity of essential oils depends mainly on the chemical structure of their components and their concentrations. In our research, undiluted lavander essential oil exhibited the best antimicrobial activity in comparation to different dilutions. According to Gavanji and coworkers (2014) in *Lavandula officinalis* oil major components are: 1,8-cineol, camphore, verbenone, alpha-pinene, thymol but Soković *et al*. (2007) recorded that linalyl acetate and linalool are the most abundant components in lavander oil. In research of Hamad *et al*. (2013) it has been demostrated that linalool and camphor were the most plentiful components of *Lavandula officinalis* oil. Essential oils containing linalol have cytotoxic activity, destroy the cell well of bacteria, inhibit the activity of bacterial enzymes, and prevent the translation of a particular gene (Park *et al*., 2012). 1,8-cineole and α-pinene are a lipophilic compounds and because of that they have greater affinity for cell membranes and greater toxicity and antimicrobial activity. For this reason, 1,8-cineole and camphor are useful substances in producing numerous drugs and have antiseptic properties (Sokovic *et al.*, 2007; Sardashti and Pourramazani, 2012).

Table 1. Antibacterial activity of *Lavandula officinalis* L. essential oil in different dilutions

Bacterial species	Inhibition zones (mm)			
	Undiluted	75%	50%	25%
Bacillus subtilis ATCC6633	8	6	6	0
Staphylococcus aureus ATCC6538P	13	10	8	0
Escherichia coli ATCC8739	14	12	10	0
Pseudomonas aeruginosa ATCC 9027	12	8	0	0
Shigella flexneri	18	14	12	8
Salmonella enteriditis	8	6	0	0
Escherichia coli	15	13	0	0
Proteus mirabillis	28	18	12	8
Klebsiella pneumoniae	12	8	0	0

In our evaluation of antibacterial potential of *T. serpyllum* essential oil, the highest antibacterial activity was observed against strains of *Salmonella enteriditis* and *Klebsiella pneumoniae* followed by *Proteus mirabillis* and however the lowest antimicrobial activity has been recorded on strain of *Staphylococcus aureus* (Table 2). It is interesting that greater inhibitory effect was recorded on clinical isolate of *Escherichia coli* than on referrent strain. The similar activity of thyme essential oil has been demonstrated by other researchers (Wesolowska *et al*., 2015). Wild thyme has excellent aroma and it is widely used as aromatic plant with antiseptic, diaphoretic, analgesic, carminative, expectorant and diuretic activity. Nikolić *et al*. (2014) found twenty nine compounds in *T. serpyllum* oil and showed that oxygenated monoterpenes are the major content observed. The main constituent of the oil was thymol, followed by carvacrol and p-cymene. Strong antibacterial effects of thymol and carvacrol were observed against Gram-positive and Gram-negative bacteria in study of other authors (Rasooli and Mirmostafa, 2002; Abed *et al*., 2014; Du *et al*., 2015; Varga *et al*., 2015). Inhibitory effects of carvacrol and thymol against microorganisms has been attributed to chemical structure of these molecules which act as proton exchangers reducing the gradient accros the cytoplasmic membrane (Rai and Chikindas, 2011). Du and coworkers (2015) recorded in their study that *E. coli* strains they used were more sensitive to thymol, while *S. enteritidis* strains were more susceptible to carvacrol, compared with other pathogens. Results of this research indicated that wild thyme essential oil had a higher antimicrobial effects than lavander oil. In both cases undiluted and 75% concentrated lavander and thyme oil had the significants antibacterial action. In our research, both tested oils were found to be inhibiting Gram-positive and Gram-negative bacteria but it can be observed that tested Gram-negative bacteria were more susceptible to investigated lavander essential oil than Gram-positive bacteria. Namely, the cell wall of Gram-negative bacteria contains an additional outer membrane that prevents the diffusion of hydrophobic components through the lipopolysaccharide bilayer (Vaara, 1992). The lipopolysaccharide layer that covers the cell wall of the Gram-negative bacteria allows them much better opacity. However, in our study it has been shown that lavander oil on the contrary, acted better on Gram-negative than on Gram-positive bacteria. This can be associated with the action of linalone as a basic component of *Lavandula officinalis* essential oil and can be explained by the synergism of various molecules contained in this etheric oil.

Table 2. Antibacterial activity of *Thymus serpyllum* L. essential oil in different dilutions

Bacterial species	Inhibition zone (mm)			
	Undiluted	75%	50%	25%
Bacillus subtilis ATCC6633	20	14	11	9
Staphylococcus aureus ATCC6538P	13	8	0	0
Escherichia coli ATCC8739	8	5	0	0
Pseudomonas aeruginosa ATCC9027	16	14	0	0
Shigella flexneri	15	11	7	0
Salmonella enteriditis	30	20	12	0
Escherichia coli	20	12	10	0
Proteus mirabillis	20	18	0	0
Klebsiella pneumoniae	22	20	0	0

CONCLUSION

From this study it can be concluded that essential oils obtained from *Lavandulla officinalis* L. and *Thymus serpyllum* L. exhibited significant antibacterial effects on different referent and clinical bacterial strains. The results of our research justified the traditional use of lavender and thyme essential oil for treating inflammation as well as an antiseptic. We believe that the present investigation together with previous studies supported the antibacterial properties of lavander and thyme oil. From all of the above, we can conclude that there is a lot of potential when it comes to active components in plants that modern medicine should investigate closely.

REFERENCES

Abed, N. E. I, Kaabi, B., Smaali, M. I., Chabbouh, M., Habibi, K., Mejri, M., Marzouki, M. N., Ahmed, S. B. H. (2014): Chemical composition, antioxidant and antimicrobial activities of *Thymus capitata* essential oil with its preservative effect against *Listeria monocytogenes* inoculated in minced beef meat. Evidence-Based Complementary and Alternative Medicine. Volume 2014.

Adam, K. L. (2006): Lavender Production, Products, Markets, and Entertainment Farms. A Publication of ATTRA: National Sustainable Agriculture Information Service, USA.

Botterweck, A. A. M., Verhagen, H., Goldbohm, R. A., Kleinjans, J., Van den Brandt, P. A. (2000): Intake of butylated hydroxyanisole and butylated hydroxytoluene and stomach cancer risk: Results from analyses in the Netherlands cohort study. Food Chem. Toxicol. *38*, 599–605. pp. 267–359, Dekker, New York, NY.

Bowles, E. J. (2004): The chemistry of aromatherapeutic oils (3rd ed.). Crows Nest, NSW: Allen & Unwin Academic.

Božin**,** B. (2009): Biohemijska i farmakološka ispitivanja vrsta roda *Allium* L. (Sect. *Allium*). Doktorska disertacija. Univerzitet u Novom Sadu, Prirodno-matematički fakultet, Novi Sad.

Burt, S. (2004): Essential oils: their antibacterial properties and potential applications in foods – a review. International Journal of Food Microbiology, 94: 223–253.

Ćavar, S., Maksimović, M., Šolić, M. E., Jerković-Mujkić, A., Bešta, R. (2008): Chemical composition and antioxidant and antimicrobial activity of two *Satureya* essential oils. *Food Chem*. 2008;111(3):648–653.

Du, E., Gan, L., Li, Z., Wang, W., Liu, D., Guo, Y. (2015): In vitro antibacterial activity of thymol and carvacrol and their effects on broiler chickens challenged with *Clostridium perfringens.* J. Anim Sci Biotechnol. 6:58.

Erenler, R., Telci, I., Uluta, M. Demirta, I., Gul, F., Elmastas, M., Kayir, O. (2015): Chemical constituents, quantitative analysis and antioxidant activities of

Echinacea purpurea (L.) MOENCH and *Echinacea pallida* (nutt.) NUTT. Journal of Food Biochemistry.

Evandri, M . G., Battinelli, L., Daniele, C., Mastrangelo, S., Bolle, P., Mazzanti, G. (2005): The antimutagenic activity of *Lavandula angustifolia* (lavender) essential oil in the bacterial reverse mutation assay. Food Chem.Toxicol., 43, 1381.

Gavanji, S., Mohammadi, E, Larki, B., Bakhtari, A. (2014): Antimicrobial and cytotoxic evaluation of some herbal essential oils in comparison with common antibiotics in bioassay condition. Integrative Medicine Research

Hamad, K. J., Al-Shaheen, S. J. A., Kaskoos, R. A., Ahamad, J., Mir, S. R. (2013): Essential oil composition and antioxidant activity of *Lavandula angustifolia* from Iraq. Int. J. Pharm;4:117–20.

Hassanpouraghdam, M. B., Hassani, A.,Vojodi, M., Asl, B. H., Rostami, A. (2011): Essential oil constituents of *Lavandula officinalis* Chaix. from Northwest Iran chemija. Vol. 22. No. 3. P. 167–171.

Höfling, J. F., Anibal, P. C., Obando-Pereda, G. A., Peixoto, I. A. T., Furletti, V. F., Foglio, M. A., Gonçalves, R. B. (2010): Antimicrobial potential of some plant extracts against Candida species. Braz. J. Biol., vol. 70, no. 4, p. 1065-1068.

Jarić, S., Mitrović, M., Pavlović, P. (2015): Review of Ethnobotanical, Phytochemical, and Pharmacological Study of *Thymus serpyllum* L. Evidence-Based Complementary and Alternative Medicine,Volume 2015.

Lahlou, M. (2004): Essential oils and fragrance compounds: bioactivity and mechanisms of action. Flavour and Fragrance Journal, 19, 159–165.

Lis-Balchin, M. (2002): *Lavender: The Genus Lavandula*, Taylor and Francis Publications, London, UK.

Madhavi, D. L., Salunkhe, D. K. (1995): Toxicological aspects of food antioxidants. In *Food Antioxidants.* In *Food Antioxidants* (D.L. Madhavi, S.S. Deshpande and D.K. Salunkhe, eds) pp. 267–359, Dekker, New York, NY.

National Committee for Clinical Laboratory Standard (2001): In Performance standards for anti-microbial susceptibility testing: 11th informational supplement. Document M100-S11, Wayne, PA.

Nikolić, M., Glamočija, J., Ferreira, I. C. F. R. (2014): Chemical composition, antimicrobial, antioxidant and antitumor activity of *Thymus serpyllum* L., *Thymus algeriensis* Boiss.and Reut and *Thymus vulgaris* L. essential oils. Industrial Crops and Products Volume 52, January 2014, Pages 183-190.

Park, S., Lim, Y., K., Freire, M.,O., Cho,E., Jin, D., Kook, J.,K. (2012): Antimicrobial effect of linalool and a-terpineol against periodontopathic and cariogenic bacteria, Anaerobe 2012; 18: 369–72.

Phongpaichit, S., Subhadhirasakul, S., Wattanapiromsakul, C. (2005): Antifungal activities of extracts from Thai medicinal plants against opportunistic fungal pathogens associated with AIDS patients. Mycoses, vol. 48, p. 333-338.

Rai, M., Chikindas M. (2011): Natural Antimicrobials in Food Safety and Quality. Wallingford, Oxfordshire, UK.

Rasooli, I., Mirmostafa, S. A. (2002): Antibacterial properties of *Thymus pubescens* and *T. serpyllum* essential oils. Fitoterapia 73:244-250.

Rostami, H., Kayemi, M., Shafiei, S. (2012): Antibacterial activity of *Lavandula officinalis* and *Melissa officinalis* against some human pathogenic bacteria. Asian Journal of Biochemistry.

Sardashti, A. R., Pourramazani, H. M. (2012): Chemical composition of the essential oil of A. kermanensis from Taftanaera by GC/MS technique. Int. J. Agric. Crop Sci 2012;4:561–3.

Sokovic, M., Marin, P. D., Brkić, D., van Griensven, LJ. L. D. (2007): Chemical composition and antibacterial activity of essential oils of ten aromatic plants against human pathogenic bacteria. Food 1:220–226

Vaara, M. (1992): Agents that increase the permeability of the outer membrane. Microbiol Rev 56, 395–411.

Varga, E., Bardocz, A., Belak, A., Maraz, A., Boros, B., Felinger, A., Boszormenyi, A., Horvath, G. (2015): Antimicrobial activity and chemical composition of thyme essential oils and the polyphenolic content of different Thymus extracts. Farmacia 63(3):357-361.

Verma, R. S., Rahman, L. U., Chanotiya, C. H . S., Verma, R., Chauhan, K., A., Yadav, A., Singh, A., Yadav, A. K. (2010): Essential oil composition of *Lavandula angustifolia* Mill. cultivated in the mid hills of Uttarakhand, India. *J. Serb. Chem. Soc.*, 75(3), 343.

Wesołowska, A., Grzeszczuk, M., Jadczak, D., Nawrotek, P., Struk, M. (2015): Comparison of the Chemical Composition and Antimicrobial Activity of *Thymus serpyllum* Essential Oils. Not Bot Horti Agrobo, 2015, 43(2):432-438.

EFFECT OF PESTICIDES ON THE YIELDS OF CLERY AND JOLY STRAWBERRY (*FRAGARIA MOSCHATA* DUCH.) VARIETIES*

Viktor József Vojnich[1], Richárd Fábián[1]

Original scientific paper

Summary

Two strawberry varieties (Clery, Joly) were examined in our research is that the effect of different types of pesticides how the yield changes. The pesticides used are: DiPel DF; Microthiol special; Switch 62.5 WG; Karate Zeon 5 CS; Zoom 11 SC. The study was conducted in a 100 meters long, 8 meters wide plastic tunnels area in Kiskőrös (Bács-Kiskun county), Hungary. In our experiment were used 3 treatments with 2 repetitions. The treatments are: 1, untreated control; 2, 75% treated with pesticides; 3, 100% treated with pesticides. The results of the experiment have shown that higher dose yields were achieved when the elevated dose of plant protection product was administered (treatment 3.). Excessive prevalence of diseases (e.g. *Botrytis cinerea*) was due not only to reduced doses of plant protection products (treatment 1. and treatment 2.), but also due to cultivation technology. To prevent *Botrytis cinerea* proposed instead of the longer foil tent, to create more, smaller ventilation and to place ventilation windows.

Key words: *Strawberry, Clery, Joly, pesticides, yield*

INTRODUCTION

Strawberries belong to the roses (*Rosaceae*) family of the *Fragaria* genus. The native wild strawberries are found in Europe, Asia, North and South America. The basic species is the forest strawberry (*Fragaria moschata* Duch.) (Galetta and Bringhurst, 1990; Papp, 1997).

The life of strawberries and performance are very large impact on the quantity and quality of the foliage, which is greatly influenced by temperature. The formation of leaves in early spring and late autumn is the slowest. The maximum progress achieved during the summer, June, July, August period, this time of 8-10 days as a new leaf grows. Generally, the development and appearance of leaves is inhibited by temperatures below 5 °C. Slower growth is observed in the canopy during the differentiation of buds (Szilágyi, 1975).

---

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] 1John von Neumann University, Faculty of Horticulture and Rural Development
H-6000, Hungary, Kecskemét, Mészöly Gyula tér 1-3.
Correspondence: Viktor József Vojnich, e-mail: vojnich.viktor@kfk.kefo.hu

The yield of strawberries largely depends on the health status of the plantation. Large, contiguous orchards cultivated strawberry plants to create all the conditions that pathogens and pests from multiplying greatly. The plant protection disadvantage that involves is high costs, but still carrying out professionally rewarding. Plant protection is not only a matter of chemical treatment, mediated methods such as good selection of crops, good agrotechnics and plant management in the right and timely manner contribute to more effective and cheaper protection against pests and pathogens. The importance of healthy propagation material deserves special emphasis. The indiscriminate and unjustified use of chemicals useful in the environment, wildlife, cause such havoc that the grower can backfire in the short term, and may be harmful because of withdrawal options. It is a very important principle that chemicals and sprays should only be spilled out when absolutely necessary (Szilágyi, 1975).
The major diseases of strawberries:
1, Diseases of virus: *strawberry crinkle virus* (SCV), *strawberry mottle virus* (SMV), *strawberry mild yellow-edge virus* (SMYEV), *strawberry vein banding virus* (SVBV), *arabis mosaic virus* (ArMV) (Glits *et al*., 1997; Glits and Folk, 2000).
2, Diseases of fungi: *Phytophthora cactorum*, *Phytophthora fragariae*, *Botrytis cinerea*, *Sphaerotheca macularis*, *Mycosphaerella fragariae*, *Diplocarpon earliana*, *Phomopsis obscurans*, *Gnomonia comari* (Glits *et al*., 1997; Glits and Folk, 2000).
<u>The most important animal pests of strawberries:</u> *Melolontha melolontha*, *Elateridae*, *Otiorhynchus ovatus, Otiorhynchus rugusostriatus*, *Aphelenchoides fragariae*, *Caenorrhinus germanicus*, *Phytonemus pallidus* (Jenser *et al.*, 1998; Glits *et al.*, 1997; Jenser, 2003).
Weeds in the strawberry plantation: Before the strawberry plantation, the area to be planted should be mainly released from perennial weeds. An effective in controlling weeds and applied agro-technical methods were of mulching cultivation as well. In this method we can weave the plantation with a multilayer layer or black foil cover. With properly implemented weed control, we can ensure that our crop grows undisturbed, thus creating the foundation for effective strawberry production. The cultivation duration can be increased, which is intended for efficiency (Kádár, 2016).
<u>The most important weeds of strawberries:</u> *Convolvulus arvensis*, *Cirsium arvense*, *Rubus caesius*, *Cynodon dactylon*, *Elymus repens*, *Stellaria media*, *Lamium spp*., *Veronica spp*., *Capsella bursa-pastoris*, *Chenopodium spp*., *Polygonum spp*., *Amaranthus spp*. (Ujvárosi, 1973; Hunyadi *et al.*, 2011).

MATERIALS AND METHODS

The experiment was set up in Kiskőrös (Bács-Kiskun County) in the strawberry plantation of 0.35 ha. The field since 2011 and going on field cultivation of strawberries in plastic tunnels. The experimental area was 600 m^2 (100 m long and 6 m wide), which was set in a foil tent.
The soil type was of sandy soil. In terms of strawberries, this is an acceptable medium because it is a loose structure with easy warming. The total salt content (EC, electrical

conductivity) was 0.02 mS. The lime content was 3.19 m / m% (preferred for strawberry cultivation, only trace amounts of calcium carbonate). The pH of the soil tested was 8.1 and the humus content was 0.385 m / m%, which is very small compared to the humus content of strawberry 1.5%.
According to Araczki and Török (1999), it is characteristic of the Kiskőrös region that it is moderately warm and dry. The annual sunshine hours reach 2025 hours. The average annual temperature was 10-11 °C. The annual average temperature fluctuation can reach 24-25 °C. The season was typical of short winter and early spring. The area is prone to rainfall, an average rainfall of 544 mm per year, but below the strawberry plantation where drainage irrigation equipment performs strawberry water supply.
The installation time was 10 August 2015. In the foil tent 6 twin-line ridge cultivation was going on, it has been a drip irrigation system and raising the ridges is dragged next to the black UV stable agro foil. The spacing was 20 cm, the line spacing was in the twin rows, relatively narrow about 25 centimeters so the number of stock in the foil tent was about 6150 strawberries.
It is practically impossible to satisfy the high water demand of strawberries without irrigation, so here is a controllable drip irrigation system with twisted, black foil-covered ridges cultivation. The advantage of this method is that it does not humidify the environment of plants so it does not enhance the potential for fungal diseases.

Characterization of strawberry varieties used in the experiment:

Clery

Place of origin: Italy. Clery perfectly adapted to the continental climate in Hungary. Medium growth, medium-density bush varieties tolerant to foliar and root diseases. Its flowers are rich in pollen and have good resistance to frost. Its fruit is evenly large, conical, bright red, very hard, with sweet good taste. Its ripening time is very early, depending on the cultivation system used 1-2 weeks before the Elsanta variety. Its fertility depends heavily on the type of propagation used. According to domestic experience, it is sensitive to the high calcareous content of the soil (Dénes, 2014).

Joly

Place of origin: Italy. The bush was growth medium strength, not too dense, upright habits. It is resistant to fungus causing leaf and root disease. Its root system is strong and therefore adapts well to drier soil conditions and is not so sensitive to fertilizer dosing. Its flowers are large, with outstanding pollen production and good pollen. The flowers are located under the foliage, on a long barrel with up to five flowers. Fruits of the whole harvest season large, cone-shaped, bright red color. The color of her meat is the same as the outside color, extremely hard, it is good for the packing and delivery trials. Joly was excellent storage and transport. The fruit is hard and very good, very sweet taste. It needs a long cold winter (Dénes, 2014).

Two strawberry varieties (Clery, Joly) were examined in our research is that the effect of different types of pesticides how the yield changes (Table 1). The pesticides used are: DiPel DF (insecticide); Microthiol special (fungicide); Switch 62.5 WG (fungicide); Karate Zeon 5 CS (insecticide); Zoom 11 SC (acaricide) (Ocskó, 2016). The pesticides used of different times (Table 2). The cultivation area was disinfected with Basamid G (soil disinfectant). In our experiment were used 3 treatments with 2 repetitions. The treatments are: 1, untreated control; 2, 75% treated with pesticides; 3, 100% treated with pesticides.

Table 1. The effect of different types of pesticides of two strawberry varieties (Clery, Joly).

Treatments	Pesticides					
	Basamid G	Microthiol special	Switch 62,5 WG	Karate Zeon 5 CS	Zoom 11 SC	DiPEL DF
(1) Untreated control	50 g/m^2	0 g/100 m^2	0 g/100 m^2	0 ml/100 m^2	0 ml/100 m^2	0 g/ 100 m^2
(2) 75% treated with pesticides	50 g/m^2	45 g/100 m^2	7.5 g/100 m^2	1.5 ml/100 m^2	3.75 ml/ 100 m^2	11.25 g/ 100 m^2
(3) 100% treated with pesticides	50 g/m^2	60 g/100 m^2	10 g/100 m^2	2 ml/100 m^2	5 ml/100 m^2	15 g/100 m^2

Table 2. The pesticides used of different times.

Time of the treatments	Pesticides					
	Basamid G	Microthiol special	Switch 62,5 WG	Karate Zeon 5 CS	Zoom 11 SC	DiPEL DF
First	20. 07. 2015	10.03. 2016	25.03. 2016	12.04. 2016	16.04. 2016	05.09. 2015
Second		30.03. 2016	04.04. 2016	26.04. 2016		15.09. 2015
Third			18.04. 2016			

RESULTS AND DISCUSSION

In the next chapter, I will describe the yield of strawberries in the experimental field. I compare crop yields with national and international strawberries yields in the previous year. So I demonstrate results, yields obtained after pesticide testing in the pilot area.

The 100% treated with pesticides (treatment 3) line installed with the Clery variety yielded the next yield after multiple picking and measurement. In the 100-meter-long line containing approximately 1,020 strawberries, 141 kg of healthy, fruitable fruit was produced (Table 3). The value was 14.1 t/ha of this converted hectare crop yield. Over the last 25 years, the average yield of domestic yields was slightly exceeded,

which was 10.6 t/ha, but still corresponds to a relatively average crop yield. The resulting yield is meant greater than 5.1 tons per hectare in value than the 2010 European average strawberry crop (9.0 t/ha) value. The nutrient solution and the pesticide handling also showed normal value.
The Joly variety experiment of treatment 3 area resulted in 153 kg (15.3 t/ha) yield. The yield of the Clery variety was lower due to the higher number of *Botrytis cinerea*.

The results of the 75% treated with pesticides in Clery (treatment 2) was 9.2 t/ha. The main cause of the decline was the *Botrytis cinerea* fungus disease, and the damage that occurred during in the autumn of moth and butterfly species in 2015.
In the same treatment at the Joly variety 103 kg of healthy fruit crops could be harvested. From the Clery variety, fewer healthy crops can be taken in the case of a reduced plant protection product than the Joly variety with the same amount of spray dosage.

The 1 treatment (untreated control), the yield of the Clery variety was only 47 kg (4.7 t/ha), while Joly was 58 kg (5.8 t/ha) yield.
Both species of aphid and mite damage also contributed to the decrease in yield.
The yield decline is the biggest cause of *Botrytis cinerea*.

Table 3. The results of Clery and Joly strawberry varieties.

Variety	Untreated control	75% treated with pesticides	100% treated with pesticides
Clery	4.7	9.2	14.1
Joly	5.8	10.3	15.3

CONCLUSIONS

Treatment 3 (100% treated with pesticides): If the normal amount of doses applied, we may still encounter some diseases and cause a decrease in yields for both varieties. The loss from loss of crop is compensated for by the fact that the cost of expensive pesticides is less and the strawberries are better sold on the market.
Treatment 2 (75% treated with pesticides): The incidence of fungal diseases was much higher than that of the 3 treatments, so the yield decrease was higher. The strawberries caused a drastic drop in yield, so I suggest increasing the dose of the Switch 62.5 WG fungus, which is already direct to fruit protection, to increase the economy and yield.
Treatment 1 (untreated control): There were high levels of fungus and insect infestation in the untreated lines of the plant protection product, so strawberries, especially under a foil or greenhouse, were not recommended without the use of plant protection products. The trapped strawberries believed to be healthy may also have traces of unseen infection that are not healthy for the human body.

The large-scale outbreaks of illnesses were due not only to reduced doses of plant protection products, but also to the cultivation technology. The 100 m long polytunnel ventilation difficult to carry out perfect, therefore propose setting up a number of smaller, less foil tent through which more easily reach the air flow, thus reducing fluid under indoor space humidity, humidity which allows the fungal infections overgrowth. The long-foil tent ventilation is not possible to place the doors at both ends, it is advisable to vent the foil tent sides of the windows.
The strawberries collected at the end of the experiment were not sold and not consumed, but the total strawberries were destroyed.

ACKNOWLEDGEMENT

This research is supported by EFOP-3.6.1-16-2016-00006 "The development and enhancement of the research potential at Pallasz Athéné University" project. The Project is supported by the Hungarian Government and co-financed by the European Social Fund.

REFERENCES

Araczki, L., Törö, Zs. (1999): Kiskőrös 2000-ben. Kaffer Kiadó, Kiskőrös, p. 8.

Dénes, F. (2014): Szamócatermesztés. Mezőgazda Kiadó, Budapest, p. 58., p. 60.

Galetta, J. G., Bringhurst, R. S. (1990): Strawberry management. In.: Galetta, J.G. – Himelrick, D.G. (ed.): Small Fruit Crop Management, Prentice Hall, Englewood Cliffs, New Jersey, pp. 83-156.

Glits, M., Folk, Gy. (2000): Kertészeti növénykórtan. Mezőgazda Kiadó, Budapest, p. 15., p. 35., pp. 263-272.

Glits, M., Horváth, J., Kuroli, G., Petróczi, I. (1997): Növényvédelem. Mezőgazda Kiadó, Budapest, pp. 592-609.

Hunyadi, K., Béres, I., Kazinczi G. (2011): Gyombiológia. Mezőgazda Kiadó, Budapest, pp. 567-568.

Jenser, G. (2003): Integrált növényvédelem a kártevők ellen. Mezőgazda Kiadó, Budapest, pp. 172-173.

Jenser, G., Mészáros, Z., Sáringer, Gy. (1998): A szántóföldi és kertészeti növények kártevői. Mezőgazda Kiadó, Budapest, pp. 20-21., pp. 147-153., pp. 183-188., pp. 232-233., pp. 243-245.

Kádár, A. (2016): Vegyszeres gyomirtás és termésszabályozás. Alföldi Nyomda Zrt., Debrecen, pp. 367-369.

Ocskó, Z. (2016): Növényvédő szerek, termésnövelő anyagok I. Reálszisztéma Dabasi Nyomda Zrt., Budapest, p. 61., pp. 192-193., pp. 326-327., pp. 395-396., pp. 599-600., p. 676.

Papp, J. (1997): Szamóca. In. Soltész M. (szerk.) Integrált gyümölcstermesztés. Mezőgazda Kiadó, Budapest, pp. 751-775.

Szilágyi, K. (1975): Szamóca. In: Sass B. (szerk.): Növényvédelem. pp. 149-150., Mezőgazdasági Kiadó, Budapest, pp. 34-42.
Ujvárosi, M. (1973): Gyomnövények. Mezőgazdasági Kiadó, Budapest. p. 13.

DETECTION AND MOLECULAR CHARACTERIZATON OF PLUM POX VIRUS ISOLATES IN PEACH ORCHARDS IN SERBIA*

DETEKCIJA I MOLEKULARNA KARAKTERIZACIJA IZOLATA VIRUSA ŠARKE ŠLJIVE U ZASADIMA BRESKVE U SRBIJI*

Darko Jevremović[1]**, Svetlana Paunović[1]

Original scientific paper

Summary

Sharka disease, caused by *Plum pox virus* (PPV) is one of the most detrimental viral diseases of plum, peach and apricot. In order to access the PPV diversity in peach orchards a total of 87 leaf samples from 36 orchards located in 15 distinct sites were collected and analyzed. Samples were tested by Immunocapture-reverse transcription-polymerase chain reaction (IC-RT-PCR) using strain-specific primers targeting C-ter NIb–N-ter CP and 6K1-CI genomic regions. PCR products were analyzed by electrophoresis in 1.5% agarose gel and stained with ethidium-bromide. As a result, 78 samples were found to be infected with PPV. In single infections, PPV-M strain was detected in 73 samples, and PPV-D in 2 samples. Mixed infections (PPV-M+PPV-D) were confirmed in 3 analyzed samples. The partial nucleotide sequence of the CP coding region was determined for 10 isolates from 8 localities. The nucleotide and amino acid sequence identity among the analyzed isolates ranged from 97.9−99.6% and 97.9−100%, respectively. Presented results indicated a strong association between PPV-M and peach in Serbia.

Keywords: *PPV, Prunus persica L., IC-RT-PCR, sequencing*

Rezime

Šarka šljive, koju izaziva virus šarke šljive (*Plum pox virus* - PPV), je jedna od najdestruktivnijih virusnih bolesti šljive, breskve i kajsije. U cilju utvrđivanja diverziteta virusa šarke u zasadima breskve analizirano je 87 uzoraka lišća sakupljenih iz 36 voćnjaka u 15 lokaliteta. Uzorci su analizirani IC-RT-PCR metodom (Immunocapture-reverse transcription-polymerase chain reaction) primenom soj-specifičnih prajmera sa ciljnim sekvencama u C-ter NIb–N-ter CP i 6K1-CI regionu genoma PPV. PCR proizvodi su analizirani elektroforezom u 1,5% agaroznom gelu i bojenjem etidijum-bromidom. Kao rezultat, kod 78 uzoraka je potvrđeno prisustvo

---

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Fruit Research Institute, Kralja Petra I 9, 32000 Čačak, Serbia
**Corresponding author: darkoj@ftn.kg.ac.rs

virusa šarke šljive. U pojedinačnim infekcijama, PPV-M soj je detektovan kod 73 uzorka i PPV-D soj kod 2 uzorka. Mešane infekcije (PPV-M+PPV-D) su potvrđene kod 3 analizirana uzorka. Parcijalne nukleotidne sekvence CP regiona genoma su determinisane kod 10 izolata poreklom iz 8 lokaliteta. Identitet nukleotidnih i sekvenci aminokiselina je kod analiziranih izolata varirao od 97.9−99.6% i 97.9−100%, respektivno. Prikazani rezultati ukazuju na jaku asocijaciju između PPV-M soja i breskve u Srbiji.

Ključne reči: *PPV, Prunus persica L., IC-RT-PCR, sekvencioniranje*

INTRODUCTION

Sharka disease, caused by *Plum pox virus* (PPV) is considered as one of the most detrimental viral diseases of stone fruits. Scholthof *et al*. (2011) grouped PPV into the "Top 10" plant viruses in molecular plant pathology based on scientific and economic importance. Since its discovery in 1932, Sharka has been considered as a calamity in plum, apricot and peach orchards (Garcia *et al*., 2014). PPV is present in Europe, Asia, North Africa, North and South America. According to some estimations, more than 100 million of stone fruit trees are infected in Europe. In highly susceptible plum varieties (e.g. Požegača) PPV causes premature fruit drop and reduces fruit quality, which can lead to total yield losses. In infected sensitive peach cultivars symptoms may appear on leaves, flowers and fruits. Symptoms are particularly evident on leaves in spring when chlorotic spots, yellow rings and lines, vein clearing, and even leaf deformation occur (Figure 1). However, in many cultivars symptoms are slightly expressed. Flowers in infected trees may exhibit color breaking mainly on cultivars with large showy flowers. Infected fruits show chlorotic spots or rings decreasing their market value (Figure 2).

Nine PPV strains (PPV-M, PPV-D, PPV-EA, PPV-C, PPV-Rec, PPV-W, PPV-T, PPV-CR and PPV-An) have been recognized so far. Three major strains (PPV-M, PPV-D and PPV-Rec) are the most dispersed and occur frequently in many European countries. Other strains are of minor importance due to their limited host preferences or geographic distribution. All three major strains have been identified in Serbia. PPV is present on plum and apricot in Serbia since mid-1930s, but the first PPV infected peach tree was detected in 1984 in north-east part of the country (Dulić & Šarić, 1986; Dulić *et al*., 1987). Since this discovery, PPV rapidly spread into many peach and nectarine orchards across the country. Peach is an important stone fruit in Serbian agriculture. According to the growing area and the production of peaches, Serbia is in the fifth place in Europe.

The aim of this study was to assess the diversity of *Plum pox virus* isolates in peach orchards in Serbia.

Figures 1 and 2. *Plum pox virus* symptoms on peach leaf and fruit

MATERIAL AND METHODS

Material

Peach (*Prunus persica* L.) leaf samples were collected during 2006 to 2014 from 15 localities: Aleksandrovac, Aranđelovac, Čačak, Jagodina, Kraljevo, Leskovac, Niš, Novi Sad, Subotica, Šabac, Užice, Valjevo, Vranje, Vršac, and Zaječar (Figure 3). A total number of 87 leaf samples were collected from 36 orchards. Asymptomatic and trees showing clear Sharka symptoms were randomly sampled from visited orchards. Each sample consisted of 20–25 leaves collected around the canopy of a tree.
Peach leaf material was tested as fresh or frozen at −20°C before further laboratory testing. Reference isolates of the PPV-M, PPV-D and PPV-Rec strains were used as controls in PCR reactions.

Figure 3. Sampling locations

IC-RT-PCR analysis

An Immunocapture - Reverse Transcription - Polymerase Chain Reaction (IC-RT-PCR) procedure was used for the detection and strain-typing of the PPV isolates. PCR plates (Sarstedt, Germany) were coated with polyclonal antibodies (0.5 mg/ml) produced at the Fruit Research Institute, Čačak. Reverse transcription was done with random hexamers using Maxima Reverse Transcriptase (Thermo Fisher Scientific, USA) according to manufacturers' recommendation. The obtained cDNA was used for further PCR analyses.

Each sample was tested in 4 PCR reactions using PPV-M and PPV-D specific primers located in the C-ter NIb–Nter-CP coding region and 6K1-CI coding region. To amplify the fragment of 467 bp located in the C-ter NIb–Nter-CP coding region primer pairs P4/P3M and P4/P3D were used (Candresse *et al*., 1998). The second set of primers CIP-M/CIP-MR and CIP-D/CIP-DR were used to amplify the fragments of 880 bp and 468 bp, located in the CI coding region, respectively (Kamenova *et al*., 2011). The cycling conditions for the PCR targeting the CP coding region were as follows: initial denaturation step at 94°C for 5 min followed by 35 cycles (92°C for 20 s, 55°C for 20 s, 72°C for 40 s) and a final extension step at 72°C for 10 min. The same conditions were used for the PCR reactions targeting the CI coding region,

except for the annealing temperature (57°C). When all 4 PCR reactions were positive, an additional PCR was performed to confirm the presence/absence of PPV-Rec using the primers mD5/mM3 encompassing the recombination breakpoint. PCR reaction was performed as described by Šubr *et al.* (2004). Isolates that gave negative results in all 4 reactions were further tested with universal primer pair P1/P2 (Wetzel *et al.*, 1991). This primer pair amplifies the 243 bp fragment from the CP coding region.
All PCR reactions were carried out in TPersonal thermal cycler (Biometra, Germany). PCR products were analyzed in 1.5% agarose gel in 0.5X TBE buffer, stained with ethidium-bromide (0.5 μg/ml) and visualized under UV light on transilluminator MacroVue UV 20 (Hoefer, USA). The presence of an amplified fragment of the expected size was considered as positive reaction. Typing of the isolates were done according to the PCR results with each specific primer pair.

Sequence analysis

To access the genetic diversity of PPV-M isolates we selected 10 isolates from 8 localities to determine the nucleotide (nt) sequence of the coat protein (CP) coding region. Selected isolates were custom sequenced in Macrogen (South Korea). Multiple sequence alignments, genetic distance estimation and phylogenetic reconstructions (neighbour-joining (NJ) method using the Kimura 2-parameter model of nucleotide substitutions) were performed using BioEdit and MEGA6 (Hall, 1999; Tamura *et al.*, 2013).

RESULTS AND DISCUSSION

To determine the PPV strain presence in positive samples RT-PCR results with specific PPV-M and PPV-D primer pairs were analyzed as described earlier by Jevremović (2013). PPV was detected in 78 out of 87 analyzed samples (89.6%). The presence of PPV was confirmed in all samples showing leaf symptoms (75 samples), but also in 3 symptomless samples.
Isolates that gave positive reaction with P4/P3M and CIP-M/CIP-MR primer pairs were characterized as belonging to PPV-M strain. In single infections, PPV-M strain was detected in 73 samples (93.6%) originating from all sampled locations. Isolates that gave positive reaction with P4/P3D and CIP-D/CIP-DR primer pairs were characterized as PPV-D. PPV-D strain was detected in 2 samples (2.6%) in single infections from locality Valjevo.
Mixed infections (PPV-M+PPV-D) were detected in 3 isolates (3.8%) from localities Novi Sad and Zaječar. To confirm the absence/presence of PPV-Rec strain in these isolates additional PCR reaction with mD5/mM3 primer pair was performed. The results confirmed the absence of PPV-Rec strain in analyzed isolates.
In 9 samples no reaction products were obtained in all 4 PCR reactions with specific primers. To verify the absence of PPV in this samples these isolates were tested with universal P1/P2 primer pair. Obtained results confirmed that the analyzed samples were not infected with PPV.

The PPV-M strain is widely present in East and Middle Europe and Mediterranean basin and is efficiently transmitted by aphids (Jevremović & Paunović, 2014). Fast spreading outbreaks are mostly associated with PPV-M, which is considered the most pathogenic PPV strain (Candresse & Cambra, 2006). Our study confirmed that PPV-M is a dominant strain in peach orchards in Serbia. Wide PPV-M distribution in peach confirms that it is well-adapted to this *Prunus* species. PPV-D was rarely found on peaches in Serbia. Comparing to PPV-M, PPV-D is described as non-aggressive strain, but may also cause epidemics in peach and other stone fruits (Dallot *et al*., 1998; Gottwald *et al*., 1995; Polák & Komínek, 2009).
Mixed infection in a single *Prunus* tree is a frequent occurrence in localities with the presence of different PPV strains and their wide distribution. The causes of mixed infections can be multiple (Jevremović & Paunović, 2015). First, planting material can be infected with one PPV strain, and then the tree is infected with another strain by aphids. Second, the use of infected reproductive material (buds and rootstocks that are infected with different PPV strains) in the process of the production of planting material. And third, the cause of mixed infections is the natural infection of a single tree with different PPV strains.
PPV-Rec (recombinant) strain is the most prevalent strain in Serbia, infecting plum, myrobalan, and apricot (Jevremović & Paunović, 2014). During previous studies on host preference of PPV strains on stone fruits in Serbia, not a single peach tree infected with PPV-Rec was found (Jevremović, 2008; Jevremović & Paunović, 2014). Until the report of Kamenova *et al*. (2011), PPV-Rec had been considered as the PPV strain unable to infect peaches. Presented results suggest that PPV-Rec strain isolates present on plum and apricot in Serbia are unable to infect peach naturally.

A 990 nt long PCR fragment of the full coat protein region was amplified from 10 isolates. All tested isolates generated a fragment of the equal size. The analysis showed that the percentage of nucleotide identity among studied PPV-M isolates in the examined region ranged from 97.9‒99.6% (mean 98.9%). The level of amino acid identity among analyzed isolates was 97.9‒100% (mean 99.1%). The high nucleotide identity of analyzed PPV-M isolates from peach is in line with earlier reports (Jevremović, 2008; Jevremović & Paunović, 2014). The mean nucleotide divergence between the analyzed isolates was low 0.0098±0.0016. Sequences of Serbian isolates were than compared with sequences of other 24 PPV-M isolates retrieved from the NCBI database. In reconstructed phylogenetic tree Serbian isolates were clustered within Mb clade with other PPV-M isolates from Serbia, Slovakia, Czech Republic and Bulgaria (Figure 4). The existence of two different PPV-M clades (Ma and Mb) and their association with geographical origin was reported earlier by Dallot *et al*. (2011).

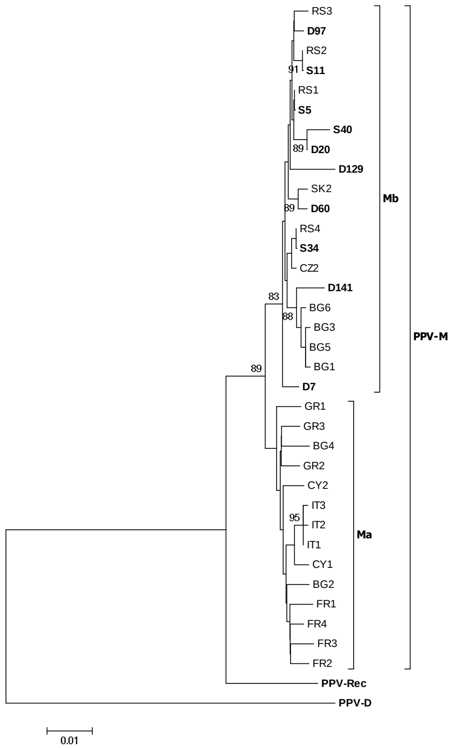


Figure 4. Phylogenetic tree reconstructed using the complete CP nucleotide sequences of 36 PPV isolates. The trees were reconstructed by NJ using the Kimura 2-parameter model of evolution. Trees were bootstrapped with 1,000 replications. Numbers at nodes indicate bootstrap values >80%. Isolates sequenced in this study are shown in bold.

CONCLUSION

Presented results confirmed the wide distribution and dominant presence of PPV-M strain in peach orchards in Serbia. PPV-D stain was detected in very low percentage in single or mixed infections in three localities. PPV-Rec strain, the dominant strain in the country, was not detected in analyzed peach samples.
Sequence analysis confirmed the high nucleotide identity of analyzed PPV-M isolates.

ACKNOWLEDGEMENT

This work has received funding from the Serbian Ministry of Education, Science and Technological Development (project TR-31064).

REFERENCES

Candresse, T., Cambra, M. (2006): Causal agent of sharka disease: historical perspective and current status of *Plum pox virus* strains. EPPO Bulletin, 36 (2): 239–246.

Candresse, T., Cambra, M., Dallot, S., Lanneau, M., Asensio, M., Gorris, M. T., Revers, F., Macquaire, G., Olmos, A., Boscia, D., Quiot, J. B., Dunez, J. (1998): Comparison of monoclonal antibodies and polymerase chain reaction assays for the typing of isolates belonging to the D and M serotypes of *Plum pox potyvirus*. Phytopathology, 88: 198-204.

Dallot, S., Labonne, G., Boeglin, M., Quiot-Douine, L., Quiot, J., Candresse, T. (1998): Peculiar plum pox potyvirus D-populations are epidemic in peach trees. Acta Horticulturae, 472: 355-366.

Dallot, S., Glasa, M., Jevremovic, D., Kamenova, I., Paunovic, S., Labonne, G. (2011): Mediterranean and central-eastern European countries host viruses of two different clades of *Plum pox virus* strain M. Archives of Virology, 156 (3): 539-542.

Dulić, I., Ranković, M., Paunović, S. (1987): Investigation of the presence and distribution of Sharka virus on peaches in Yugoslavia. Zaštita Bilja, 38 (4): 381-385. (in Serbian)

Dulić, I., & Šarić, A. (1986): Outbreak of *Plum pox virus* on peaches in Yugoslavia. Acta Horticulturae, 193: 161–165.

Garcia, J. A., Glasa, M., Cambra, M., Candresse, T. (2014): *Plum pox virus* and Sharka: A model *Potyvirus* and a major disease. Molecular Plant Pathology, 15 (3): 226-241.

Gottwald, T. R., Avinent, L., Llacer, G., Hermoso de Mendoza, G., & Cambra, M. (1995): Analysis of the spatial spread of Sharka (*Plum pox virus*) in apricot and peach orchards in eastern Spain. Plant Disease, 79: 266-278.

Hall, T. A. (1999): BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic acids symposium series, 41: 95-98.

Jevremović, D. (2008): Molecular characterization and genetic structure of *Plum pox virus* isolates in Serbia. (Master's thesis), University of Belgrade, Belgrade, Serbia. (in Serbian)

Jevremović, D. (2013): Distribution of PPV-D and PPV-Rec strains of *Plum pox virus* in Serbia and the dynamics of their spread in plum orchard. (PhD thesis), University of Belgrade, Belgrade, Serbia. (in Serbian)

Jevremović, D., Paunović, S. (2014): *Plum pox virus* strains: Diversity and geographical distribution in Serbia. Pesticidi i fitomedicina, 29 (2): 97-107.

Jevremović, D., Paunović, S. (2015): Detection and molecular characterization of *Plum pox virus* isolates in apricot orchards in municipality of Čačak. Voćarstvo, 49 (189-190): 49-55. (in Serbian)

Kamenova, I., Dallot, S., Bozkova, V., Milusheva, S. (2011): First report of the *Plum pox virus* recombinant strain on peach in Bulgaria. Plant Disease, 95 (10): 1320-1320.

Polák, J., Komínek, P. (2009): Distribution of *Plum pox virus* strains in natural sources in the Czech Republic. Plant Protection Science, 45 (4): 144-147.

Scholthof, K. B., Adkins, S., Czosnek, H., Palukaitis, P., Jacquot, E., Hohn, T., Hohn, B., Saunders, K., Candresse, T., Ahlquist, P., Hemenway, C., Foster, G. D. (2011): Top 10 plant viruses in molecular plant pathology. Molecular Plant Pathology, 12 (9): 938-954.

Šubr, Z., Pittnerová, S., Glasa, M. (2004): A simplified RT-PCR-based detection of recombinant *Plum pox virus* isolates. Acta Virologica, 48: 173-176.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. (2013): MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30: 2725-2729.

Wetzel, T., Candresse, T., Ravelonandro, M., Dunez, J. (1991): A polymerase chain reaction assay adapted to *Plum pox potyvirus* detection. Journal of Virological Methods, 33: 355-365.

INVESTIGATION OF FIRE BLIGHT (*Erwinia amylovora* (Burrill) Winslow *et al.*) APPEARANCE IN CENTRAL BOSNIA*

Azra Sito[1], Marko Runjic[2], Arnela Okic[1*] Elda Vitanovic[2]

Original scientific paper

Summary

Fire blight, caused by *Erwinia amylovora* (Burrill) Winslow *et al*., is considered as the most serious pear and apple disease in many countries around the world. This bacterial disease is included in the EPPO A2 and the EU Annex II/A2 lists, but also many other (non-EU) countries quarantine lists list it in order to prevent further spread. The fire blight causes typical tissue necrosis and in optimal conditions this can lead to complete plant destruction. Destruction degree depends on plant sensitivity to *E. amylovora.* In Bosnia and Herzegovina fire blight was confirmed on pear and individual apple trees back in 1990. Beside this fact, there is relatively little data about its distribution in the country. Although, typical symptoms simplify disease identification by visual inspection, for a precise diagnose and presence confirmation of this quarantine pathogen, laboratory tests are necessary. The aims of the research were to investigate the appearance of fire blight in central Bosnia, and to contribute in regional monitoring program of disease distribution. During the 2012 samples with fire blight symptoms were collected from several apple orchards located in central part of Bosnia and Hercegovina. After seeding on culture media, three samples with typical colonies were tested for the *E. amylovora* presence by methods according to EPPO diagnostic standards PM 7/20 (2). Commercial varieties (‘Idared’ and ‘Golden delicious’) were positive on applied nutritional and enzymatic identification tests, while the local variety ‘Senabija’ was bacteria free. The results of this research confirm fire blight appearance in central Bosnia (Sarajevo Canton).

Keywords: *bacterial disease, Erwinia amylovora, fire blight, apple, quarantine lists*

INTRODUCTION

Fire blight represents one of the most important apple diseases in the world. It is caused by bacteria *Erwinia amylovora*, which belongs to most widespread and economically most significant bacterial phytopathogens. Huge damages caused by this

---

*Rad prezentiran na 25. Međunarodnom naučno-stručnom kongresu poljoprivrede i prehrambene industrije/ Paper presented at 25th International Scientific-Expert Congress on Agriculture and Food Industry, 25-27 September 2014, Izmir, Turkey
1 University of Sarajevo, Faculty of Agriculture and Food Sciences, Department of Plant Protection, Bosnia and Herzegovina
2 Institute for Adriatic Crops and Karst Reclamation, Department of Applied Science, Split, Croatia
*Corresponding author: aokic.ppf@gmail.com

bacteria have been recorded all around the world, which led to massive losses of apple and pear orchards. The production of these fruits was entirely withdrawn.
Fire blight symptoms are characteristic and manifest in a form of inflorescence's necrosis and misplaced fruits, shoot blight, branches necrosis and drying, and in the end tree dying (Van der Zwet and Keil, 1979). As opposed to other phytopathogenic bacteria which induce creation of necrosis on plant tissue, *E. amylovora* spreads exceptionally fast from the place of a primary infection. On sensitive varieties under favorable conditions (including climate and tree physiology), disease migrates from the infected flower to the root, causing death of the host plant in one vegetation season (Vaneste and Eden-Green, 2000).
It was first described in USA at the end of 18th century but at that time the symptoms were considered to be caused by various phytopathogenic fungi, insects and even weather conditions, up until 1883 when the bacterial nature was confirmed by Burill (Van der Zwet and Keil, 1979).
The 20th century represents the time of intensive disease expansion. In Europe it was first found in Great Britain in 1957, afterwards a permanent spread of the disease was assessed throughout Europe. In 1998, with the exception of Portugal, all member countries of the European Union had fire blight on pears, apples and ornamentals (CABI, 2017) in various phytosanitary statuses (widespread, localized to restricted spots and local eradication). Even though, some wide areas of Europe remain free of fire blight, like in Italy, Spain and the south-east of France (CABI, 2017). On the territory of former Yugoslavia, this vicious pathogen was registered at the end of 1989 in Macedonia and its occurrence was officially confirmed in 1990 (Arsenijevic *et al*., 1991). The same authors also indicate that in the same year, presence of fire blight was reported in northern part of Bosnia and Herzegovina, in the district Bosanska Gradiska on pears and individual apple trees. The fact that first reported case of fire blight was on a relatively large area of about 288 ha (Panic and Arsenijevic, 1993) indicates that pathogen was present there for a long time, and that it was officially recorded. The exact data about the fire blight distribution in Bosnia and Herzegovina are limited according to Balaz *et al*. (2013), although focus on disease spread in the country was great. Trkulja *et al*. (2004) pointed out that there was a 10 year period of fire blight monitoring on the territory of Republic of Srpska where disease outbreak due to favorable weather conditions was observed in 2000. Further on, the same authors state that in 2003 a monitoring project was also set up in Federation of Bosnia and Herzegovina, financed by the national authorities. Despite all this efforts, E. *amylovora* distribution data are still incomplete.
Considering that apple has the major role in overall fruit production in Bosnia and Herzegovina, and that it is the most important fire blight host, over the last few years huge attention was shifted to disease monitoring in this country. It is necessary to mention that there are certain differences between apple varieties and disease resistance, so one of the key measures for fire blight controlling should be choosing more resistant varieties (Van der Zweit and Keil, 1979). In Bosnia and

Herzegovina a significant number of apple indigenous varieties is preserved, which are potentially tolerant to fire blight.
The aim of this research was to investigate the fire blight appearance in central Bosnia (Sarajevo Canton) and to contribute in regional disease monitoring program.

MATERIALS AND METHODS

Research was conducted in area with possible fire blight presence and infection in Sarajevo County. In June 2012, samples of four different apple varieties were collected from three different locations. First location (A) was apple orchard in Butmir (Ilidza municipality) where 'Golden delicious' variety symptomatic samples (S1) were taken. For second location (B), an orchard in Sovrle (Ilijas municipality) was chosen. In that location the symptomatic samples of commercial varieties 'Idared' (S2) and 'Golden delicious' (S3) were taken. Third location (C) was an orchard in Semizovac (Vogosca municipality), where symptomatic samples of indigenous variety 'Senabija' (S4) were taken. Also, 10 samples of plant material, such as branches, leaves, flowers and fruits, were taken for each variety. All samples showed bacterial disease symptoms.
Samples were delivered and analyzed in Federal Agricultural Bureau's laboratory in Sarajevo. It is necessary to note that for the assignment purposes considerably larger amount of samples was covered. On the remaining samples either no disease and bacteria indicators were observed, or symptoms similar to fire blight were caused by other diseases or insects. Those samples were rejected from the beginning and were not analyzed.
Within this research, four samples which showed symptoms of infection by *E. amylovora* were analyzed. Analysis was conducted in accordance with EPPO standards PM 7/20 (2) for fire blight (EPPO, 2013), which implied the method of planting on nutrient grounds and review with the help of biochemical tests. Samples weighing 0.5 g were submitted to extraction with PBS buffer and put on MULTI-Vortex V-32. After extraction, the isolation of bacteria on nutritive grounds was made. For isolating *E. amylovora*, King B (King *et al.*, 1954) and Levan nutritive grounds were used.
After formation of colonies, identification started by using various nutritive and enzyme tests. Identification was made by citrate utilization test, gram staining, indole test, Kovacs oxidase test, fluorescent pigment on King B's surface test, pathogenicity test and a final identification was made with the help of ELISA-test (EPPO, 2013). Citrate utilization test (citric acid) was made using Simmons Citrate Agar to which colonies that formed on Levan ground were transferred. Incubation was made on a temperature of 37^0C in a period of three days. Gram staining test is a quick method for diversifying gram negative and gram positive bacteria, and it involves bacteria solubility test in 3% KOH. Isolated colonies pathogenicity was tested on immature pear fruits ('Viljamovka' variety), through inoculation of bacterial suspension in accordance with EPPO standards PM 7/20 (2) for fire blight (EPPO, 2013). For final

identification DAS-ELISA was used. Commercial anti-serums specific for *E. amylovora* (Bioreba) were used. The entire procedure was made according to guidelines of PM 7/101 (1) *ELISA tests for plant pathogenic bacteria* (EPPO, 2010) and by manufacturer's protocol.

RESULTS AND DISCUSSION

Fire blight monitoring in apple orchards has been conducted in nine municipalities of Sarajevo Canton during flowering season. In three out of nine municipalities, orchards with bacterial disease symptoms were found, but without typical fire blight 'Shepherd's crook' symptom on shoots. Plant materials for *E. amylovora* laboratory analyzes were sampled in mentioned orchards. The absence of this typical symptom was due to sampling time, since they develop later in the season. Therefore, in early season, the visual symptoms of fire blight are hard to distinguish from other bacterial infection.

Bacteria colonies appeared 24 h after isolation on nutritive King B ground (Fig. 1) for three samples (S2, S3 and S4). Sample S1 was excluded from further tests, since it lacked the production of bacterial colonies even after repeated isolation procedures. Produced colonies of the other samples were creamy white, round-shaped (Yaich *et al*., 2011), and had a tendency of expansion that do not fluorescence on 366 nm under UV light after 48 h. Listed colonies were morphologically typical for *E. amylovora* on King B ground and all of the samples were assessed positive. On Levan, colonies of bacteria typical for this media by appearance and color were obtained on all analyzed samples (Fig. 2). After positive isolation, further test were made for identification purposes were made. Citrate usage test showed positive on all three analyzed samples, which could be noticed by the appearance of characteristically yellow color (Fig. 3). Gram test confirmed the bacteria negative, which is characteristic of *E. amylovora*. Also, *E. amylovora* has a negative reaction on Indole test (Fig. 4), oxidase test (Fig. 5) as well as fluorescent pigment on King B media (Fig. 6). After all conducted biochemical test, the bacteria *E. amylovora* was confirmed on two analyzed samples (S2 and S3). Conducted test review is presented in Table 1.

Table 1. Results of analyzed samples

Sample	Location	Apple variety	Isolation on nutritive media	Indol test	Gram test	Kovac's test	Citrat utilization	Fluorescent pigment on KING B media	Pathogenicity test	ELISA test
S1	A	Golden delicious	/	/	/	/	/	/	/	/
S2	B	Idared	+	-	-	-	+	-	+	+
S3	B	Golden delicous	+	-	-	-	+	-	+	+
S4	C	Senabija	+	-	-	-	+	+	-	-

The pathogenicity tests of isolated bacterial colonies were conducted on immature pear fruits where necrotic and ringed spots appear around sting place with bacterial exudates 3-4 days after incubation, if infected with *E. amylovora*. This specific symptom was recorded on S2 ('Idared') and S3 ('Golden Delicous'), whereas S4 ('Senabija') showed necrosis symptoms without exudate appearance. Since the absence of exudate drops is typical for the pathogenic bacteria *Pseudomonas syringae* (Arsenijevic, 1988), it was considered that the mentioned bacteria, which has similar biochemical properties to *E. amylovora*, could be the causal agent of symptoms described on 'Senabija' in the orchard. Final identification was made by DAS-ELISA method (Fig. 7). With this test, samples S2 and S3 were tested positive on *E. amylovora* infection.

The autochthonous variety was proved to be free of fire blight infection. Ognjanov (2005) points out that some indigenous species have a high level of horizontal, racially unspecific resistance towards causal agents of economically most significant diseases - apple scab, powdery mildew and fire blight. Therefore, the autochthonous apple variety 'Senabija' could carry some useful traits and could possibly be included in apple breeding programs. For objective and official data a considerably higher number of samples from indigenous apple assortment should be tested, but these first results impose a potential.

The conducted research shows that visual symptoms for early fire blight identification are not always reliable, since they first appear on flowers and are hard to distinguish from other bacterial infections. In this research, symptoms which were observed in the orchard on variety 'Senabija' flowers could be linked to *Pseudomonas sp* infection. The absence of exudate on immature pear fruit during the pathogenicity test and later on the negative ELISA test on these isolates, clearly indicate that.

Favorable weather conditions for fire blight infection were present on all locations. The early observed symptoms under these favorable weather conditions indicate that an early forecast system could be a benefit for control measure against fire blight. In Croatia the forecasting model MARYBLYT and BIS showed satisfactorily results (Cvjetkovic *et al*., 1999). These and similar models could be adapted in the forecast system VIPS (norv. Varsling Innen PlanteSkadegjørere – pest and plant disease notification) in Bosnia and Herzegovina (Okic *et al*., 2015).

CONCLUSION

Regarding the results of this study we may conclude that fire blight is present in Sarajevo Canton, in central Bosnia. *E. amylovora* was confirmed on two analyzed samples. Commercial varieties 'Idared' and 'Golden delicious' were positive on applied nutritional and enzymatic identification tests, while the local variety 'Senabija' was bacteria free. These findings may indicate that commercial apple varieties 'Idared' and 'Golden delicious' have a higher level of fire blight infection than autochthonous varieties, and it is necessary to conduct a large scale research to further investigate their tolerance level. Visual symptoms early in the season are not a reliable tool for fire blight identification, since they can be confused with other bacterial infections. It is important to note that results of this research would contribute to regional disease monitoring program, and a better understanding of fire blight spreading in Bosna and Hercegovina.

REFERENCES

Arsenijevic, M. (1988): Bakterioze biljaka, Naucna knjiga, Beograd, pp. 1-463.

Arsenijevic, M., Panic, M., Antonijevic, D. (1991): Fire blight of pomaceous fruit trees in Yugoslavia. Zastita bilja, Vol. 43(196), pp. 87-97.

Balaz, J., Grahovac, M., Radunovic, D., Ilicic, R., Krstic, M. (2013): The Status of *Erwinia amylovora* in the former Yugoslav Republics over the past two decades, Pestic. Phytomed. (Belgrade), Vol. 28 (1), pp. 9-22.

Bonn, W. G., Van der Zwet, T. (2000): Distribution and economic importance of fire blight. In: Fire Blight, The Disease and its Causative Agent *Erwinia amylovora* (Ed. Vanneste J.), pp. 37–53. CAB International, Wallingford (GB).

CABI (2017): *Erwinia amylovora* (original text by J. Vanneste). In: Invasive Species Compendium. Wallingford, Uk: CAB International. www.cabi.org/isc.

Cvjetkovic, B., Halupecki, E., Spoljaric, J. (1999): The occurrence and control of fire blight in Croatia. Acta Horticulturae, Vol. 489, pp. 71-77.

King, E. O., Ward, M., Raney, D. E. (1954): Two simple media for the demonstration of pyocyanin and fluorescein. Journal of Laboratory and Clinical Medicine, Vol. 44, pp. 301–307.

OEPP/EPPO (2010): PM 7/101 (1) *ELISA tests for plant pathogenic bacteria*, Bulletin, PM 7/101 Vol. 40, pp. 369 – 372.

OEPP/EPPO (2013): *Erwinia amylovora*, Bulletin, PM 7/20 (2), Vol. 43 (1), pp. 21–45.

Ognjanov, V. (2005): Autohtone sorte jabuke kao donori otpornosti na parasite. Poljoprivredni fakultet, departmant za vocarstvo i vinogradarstvo, Novi Sad, pp. 127-131.

Okic, A., Grahic J., Kurtovic M., Konjic, A., Bajric, M., Kanlic, K., Haseljic, S., Gasi F. (2015): First experiences on the open source technology platform VIPS in Bosnia and Herzegovina. Works of the Faculty of Agriculture and Food Sciences University of Sarajevo, Vol. 66/1, pp. 312-316.

Panic, M., Arsenijevic, M. (1993): Outbreak, spread and economic importance of fire blight pathogen (*Erwinia amylovora*) in Yugoslavia. Acta Horticulturae, Vol. 338, pp. 89-96.

Trkulja, V., Stojcic, J., Peljto, A., Numic, R. (2004): Prisustvo, znacaj i opasnost od daljeg sirenja *Erwinia amylovora* – prouzrokovaca bakteriozne plamenjace jabucastih vocaka u Bosni i Hercegovini. In Simpozijum o zastiti bilja u BIH. Zbornik rezimea, Sarajevo, pp. 26-28.

Van der Zwet, T., Keil, H. L. (1979): Fire blight: A bacterial disease of rosaceous plants. Agriculture Handbook Number 510, United States Department of Agriculture, Washington D. C., USA, pp. 1-200.

Vanneste, J. L., Eden-Green, S. (2000): Migration of *Erwinia amylovora* in host plant tissues. In: Vanneste J. L. (ed.). Fire Blight, the disease and its causative agent *Erwinia amylovora*. CABI Publishing, UK, pp. 73-85.

Yaich, M., Fatmi, M., Bougsiba, M., Valentini, F., Scuderi, G., D'onghia, A. M., Cirvilleri, G. (2011): Fire blight (*Erwinia amylovora* (Burrill) Winslow) in Morocco: importance, geographical distribution and characterization. Phytopathologoa Mediterranea, Vol. 50, pp. 212−227.

Figure 1. Bacteria colonies on King B ground (photo: Sito, A.)

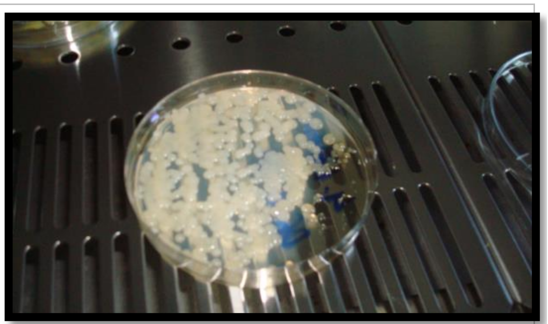


Figure 2. Bacteria colonies on Levan ground (photo: Sito, A.)

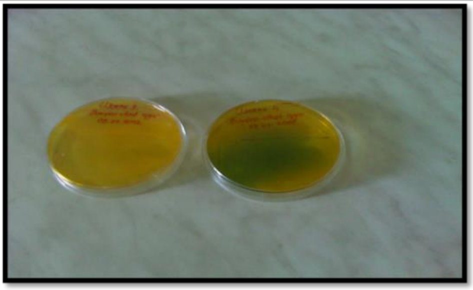


Figure 3. Citrate test - characteristically yellow color (photo: Sito, A.)

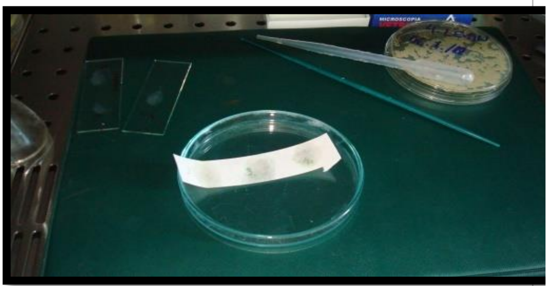


Figure 4. Indole test results (photo: Sito, A.)

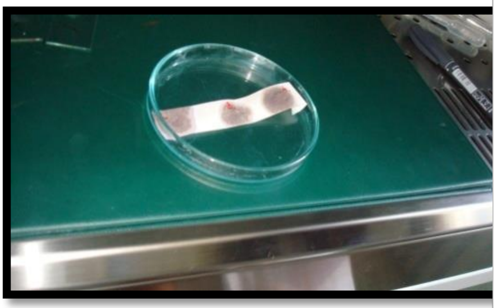


Figure 5. Oxidase test results (photo: Sito, A.)

Figure 6. Fluorescent pigment on King B ground (photo: Sito, A.)

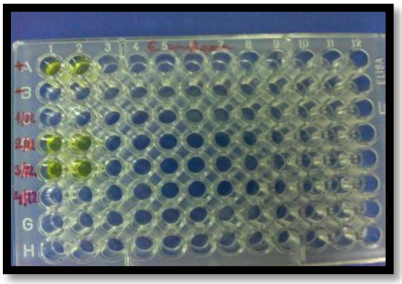


Figure 7. ELISA test results (photo: Sito, A.)

DISTRIBUTION OF JIMSONWEED (*Datura stramonium* L.) IN THE CITY OF SARAJEVO *

RASPROSTRANJENOST TATULE (*Datura stramonium* L.) NA PODRUČJU GRADA SARAJEVA

Mirha Điki ć[1], Nijaz Suljić[1], Nermina Sarajlić[2], Drena Gadžo[1]

Original scientific paper

Summary

Jimsonweed (*Datura stramonium* L.) is a strong-scented annual plant of Solanaceae family. The stem is erect, branched, with smooth toothed leaves and single, trumpet-shaped white flowers. The fruit is egg-shaped spiny capsule, filled with small, black seeds. It is native to Central America but was introduced in Europe before 1650 and became naturalized in many warm and moderate regions. It is often found along roadsides, wastelands, garbage dumps, but also in parks, gardens and other sites on nitrogen-rich soils. The entire plant is highly toxic, posing a threat to children and pets in parks and schoolyards, but is also among troublesome invasive alien species that releases allelochemicals to the environment, suppressing the development of native plants. The literature sources on this species in the area of the city of Sarajevo are very scarce, so the aim of this paper is to record the sites where jimsonweed is present, in order to be able to monitor its populations in the future and identify the most infested areas of the city in which the eradication measures should be undertaken in order to prevent further invasion of this species.

Key words: *Datura stramonium, invasive species, distribution, Sarajevo*

Rezime

Tatula (*Datura stramonium* L.) je jednogodišnja biljka iz porodice Solanaceae, koja se odlikuje jakim i neugodnim mirisom. Ima uspravnu razgranatu stabljiku, krupne i glatke listove sa nazubljenim rubom i pojedinačne, krupne bijele cvjetove. Plod je jajolika kapsula u kojoj se nalaze brojne crne sjemenke. Ova biljka vodi porijeklo iz Srednje Amerike, a u Evropu je unesena prije 1650. godine i spontano se raširila u područjima sa toplom i umjerenom klimom. Često se nalazi uz puteve, na

---

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Faculty of Agriculture and Food Sciences, University of Sarajevo, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina
[2] Faculty of Natural Sciences and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia
Correspondence: Prof. dr. Mirha Đikić, Faculty of Agriculture and Food Sciences, University of Sarajevo, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina m.djikic@ppf.unsa.ba

smetljištima, ali i u parkovima, vrtovima i drugim tlima bogatim azotom. Cijela biljka je vrlo otrovna, pa predstavlja opasnost za djecu i kućne ljubimce u parkovima, igralištima i školskim dvorištima. Ujedno je i problematična invazivna korovska vrsta koja, zbog lučenja alelohemikalija, usporava razvoj autohtone flore. U literaturi nema mnogo podataka o prisustvu ove vrste na području grada Sarajeva, pa je cilj ovoga rada da se da pregled lokacija na kojima je tatula zabilježena, kako bi se u budućnosti njene populacije mogle pratiti, ali i da se identifikuju dijelovi grada u kojima je ova vrsta najviše prisutna i u kojima treba poduzeti mehaničke i druge mjere kako bi se spriječilo njeno daljnje širenje.

Ključne riječi: *Datura stramonium, invazivne vrste, distribucija, Sarajevo*

INTRODUCTION

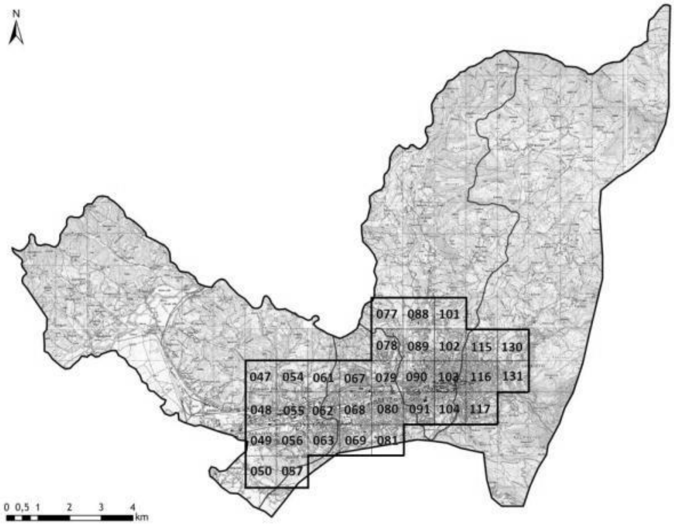
Jimsonweed (*Datura stramonium* L., Solanaceae) is a strong-scented annual plant whose region of origin is not known with certainity, but is most likely native to Asia (Mahnaz *et al*., 2011) or Central America (vanKleunen *et al*., 2007; Richardson *et al*., 2007) and is today present as an alien invasive plant species in almost all temperate and tropical regions of the world (Henderson, 2001). *Datura stramonium* is herbaceous, branched and glabrous plant that can grow up to 1.5 m tall. The leaves are hairy, large, simple dentate, oval glabrous, stalked and pale green. The flowers are solitary, large (7-10 cm long) white and trumpet-shaped. The fruits are thorny capsules, initially green, becoming brown with maturity when they divide into four segments to release the seeds. A single fruit may contain up to 650 seeds (Fatoba *et al*., 2001; Steenkamp *et al*., 2004; Richardson *et al*., 2007). *Datura stramonium* is propagated by seeds, which usually germinate in 3 to 6 weeks at 15 °C and can remain viable in the soil up to 3.5 years (Kojić & Janjić, 2000). This plant mainly inhabits recently disturbed sites in agricultural fields, industrial areas, home gardens and roadsides with nitrogen-rich soils (Đelić *et al*., 2011).

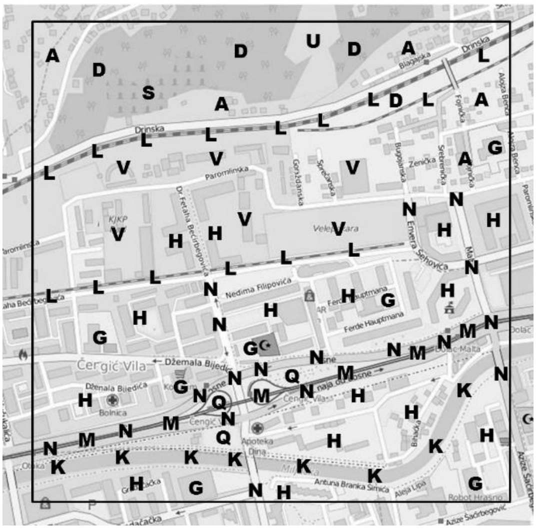
Due to its toxic properties, *Datura. stramonium* is known under different names, including Angel’s trumpet, Devil's trumpet, Devil's weed, Thorn apple, Stinkweed, Locoweed, Devil's cucumber and Hell's Bells (Mahnaz *et al*., 2011; Oseni *et al*., 2011). It has been reported that sixty-four tropane alkaloids have been detected in *Datura stramonium* plant, whereby the highest alkaloid concentration being found in seeds (Maibam *et al*., 2011). Children have a special susceptibility to atropine toxicity; even a small amount may produce severe central nervous system manifestations. So far, several authors have reported poisoning by intentional (Arouko *et al*., 2003; Spina & Taddei, 2007; Diker *et al*., 2007; Kakkar *et al*., 2015) or accidental (Chang *et al*., 1999; Wiebe *et al*., 2008; Bontoyan, 2010; Bouziri *et al*., 2011; Şanlidağ *et al*., 2014) ingestion of seeds or other parts of the plant by both children and adults. *Datura stramonium* also expresses the allelopathic effect on several species, reducing the yield of crops, especially corn (Oljača *et al*., 2002; Šćepanović *et al*., 2008) and biodiversity of local flora.

So far, there are no detailed data on distribution of Jimsonweed in the city of Sarajevo, so it was impossible to follow the dinamics of its spread and development over past years.
The aim of this paper is to map populations of *Datura stramonium* in urban area of city of Sarajevo in order to ensure its monitoring and control in the future.

MATERIALS AND METHODS

The survey was conducted in urban green spaces of city of Sarajevo, on the area of 32 km^2 (Figure 1) during vegetation period (May to October) of 2015 and 2016, on 25 habitat types (Figure 2) determined in the city (A - Home gardens; B – Forests; C – parks, lanes, plantations, orchards, nurseries; D – Forest edges, thickets and hedges in the city; E – Landfills (waste, compost, sand, timber…); F – Agricultural land; G – Public buildings and sports grounds; H – Blocks of residential buildings; I – Cracks in the concrete; J – Flower boxes; K – Water bodies (riverbanks, lakes, pools); L – Railway; M – Tramway; N – Roads (parking sites, alley trees); O – Abandoned buildings and ruins; P – Archaeological sites; Q – Public lawns and flowerbeds; R – Wall along the riverbed; S – Cemeteries; T – Cracks in natural rocks; U – Natural grasslands; V – Industrial areas; X – Construction sites; Y - Forest edges, thickets and hedges outside the city; Z – Zoo). The research was conducted by point square method (0.25 m^2), and the coverage was assessed by modified Braun-Blanquet (1965) method (1: <5% plants per square; 2: 6 – 25% plants per sqare; 3: 26 – 50% plants per square; 4: >50% plants per square).





Figures 1 and 2. Surveyed area and distribution of habitat types in a single surveyed 1 km^2 square

RESULTS AND DISCUSSION

During this research, *Datura stramonium* L. was found in 12 out of 32 surveyed 1 km^2 squares in the urban area of city of Sarajevo (Figure 3), mostly in the parts of the city with private houses and places where the construction works recently took place.

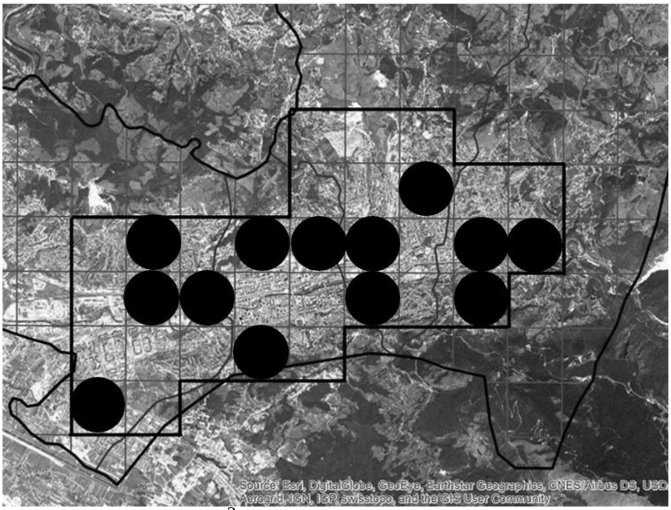


Figure 3. Parts of Sarajevo (1 km^2 squares) where *Datura stramonium* was registered in 2016 and 2017

In most locations, the plants were solitary, meaning that the overall coverage in surveyed 1 km^2 squares was 1 (less than 5% of *Datura stramonium* plants present in surveyed 0.25 m^2 squares). In three 1 km^2 squares, located in peripheral parts of the city, groups of 2-3 plants growing together were registered, so the overall coverage of *Datura stramonium* plants was assessed to 2 (i.e. 6 – 25% plants per 0.25 m^2 sqare) (Figure 4). This was the case in the areas with numerous private houses, most of which had neglected gardens.

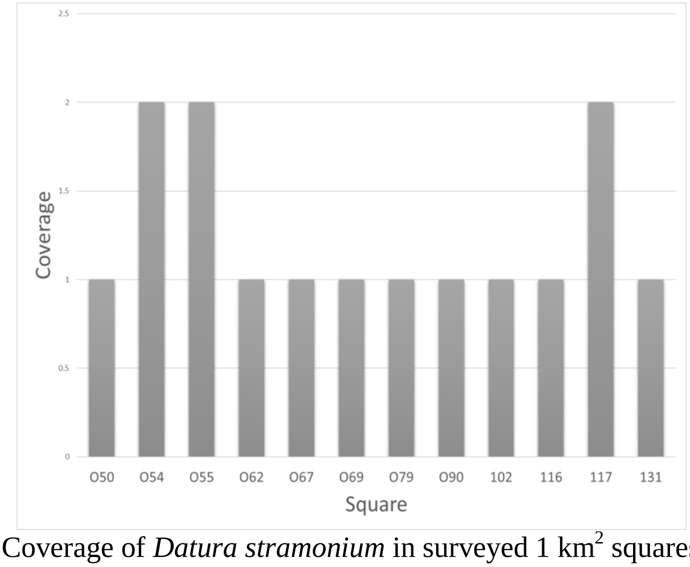


Figure 4. Coverage of *Datura stramonium* in surveyed 1 km^2 squares of city of Sarajevo

Although they were not registered in large groups in any surveyed locations, most plants were robust, well-developed and up to 80 cm tall. Only in one site in the Dobrinja neighbourhood, there were 13 young plants growing together in a rather dense group near the newly-built road. However, it should be mentioned that the eastern part of the city was not sufficiently explored, because of the fact that most small family houses there were surrouded by tall walls and fences, so it is likely that *Datura stramonium* is even more common then this research shows.
During this research, *Datura stramonium* was registered in 9 habitat types in the city of Sarajevo. It was most common in disturbed sites: home gardens and agricultural land around them, construction sites and green spaces along roads. Some plants were registered in landfills, public flowerbeds and along the railway. Individual plants were also recorded in the area of the city's zoo, in a flowerbox near the entrance to one of the city's elementary schools, and in the immediate vincinity of one of the public playgrounds, where they pose the direct threat to pets and young children.
According to Konstatinović *et al.* (2004**)**, *Datura stramonium* plants tend to express resistance to herbicides, so it is advisable to perform physical eradication measures of this invasive alien species, mostly mowing and destroying plants before flowering, in order to prevent its further spread.

CONCLUSIONS

During this research, *Datura stramonium* L. was found in 12 out of 32 surveyed 1 km^2 squares, on 9 out of 25 habitat types in the city of Sarajevo. It was most common in disturbed sites: home gardens and agricultural land around them, sites where the construction works were in progress or have recently took place, green spaces along roads, landfills, public flowerbeds and along the railway. In most locations, the plants were solitary, or in smaller groups, but robust, well-developed and up to 80 cm tall. Several plants were registered in green areas of the city's zoo, in a flowerbox near the entrance to one of the city's elementary schools, and in the immediate vincinity of one of the public playgrounds, where they pose the direct threat to pets and young children. Since this species tends to express resistance to herbicides, so it is advisable to perform physical eradication measures in order to reduce its presence in the urban green areas and prevent its further spread.

REFERENCES

Arouko, H., Matray, M. D., Bragança, C., Mpaka, J. P., Chinello, L., Castaing, F., Bartou, C., Poisot, D. (2003): Voluntary poisoning by ingestion of *Datura stramonium*. Another cause of hospitalization in youth seeking strong sensations. Ann Med Interne.154: 46–50.

Bontoyan, W. (2010): Jimsonweed Poison Associated with homemade stew - Maryland 2008. Centers for Disease Control and Prevention – Morbidity and Mortality Weekly Report. 59(4): 102–103.

Bouziri, A., Hamdi, A., Borgi, A., Bel Hadj, S., Fitouri, Z., Menif, K., Ben Jaballah, N. (2011): *Datura stramonium* L. poisoning in a geophagous child: A case report. Int J Emerg Med. 4: 31.

Braun-Blanquet, J. (1965): Plant sociology – The study of plant communities. Hafner Publisching Company, New York.

Chang, S. S., Wu, M. L., Deng, J. F., Lee, C. C., Chin, T. F., Liao, S. J. (1999): Poisoning by Datura leaves used as edible wild vegetables. Vet Hum Toxicol. 41: 242–245.

Diker, D., Markovitz, D., Rothman, M., Sendovsk, U. (2007): Coma as a presenting sign of *Datura stramonium* seed tea poisoning. Europ. Jour. of Inter. Med. 18(4): 336–338.

Đelić, G., Gutman, I., Marković, A. (2011): Najotrovnije biljke naših krajeva – tatula. Hemijski pregled, 52 (3): 58-61.

Kakkar, A., Tiwari, B., Kumar, S. (2015): Intoxication due to *Datura stramonium* in an adolescent: A case report. Rama Univ. J. Med Sci 1(2): 74-76.

Konstatinović, B., Maseldžija, M., Šunjka, D., Konstatinović, B. (2004): Ispitivanje rezistentnosti tatule (*Datura stramonium* L.) na inhibitore acetolaktatsintaze (ALS). Pesticidi i fitomedicina 19: 105-110.

Mahnaz, A., Hamid, K., Reza, A. (2012): Acute *Datura stramonium* poisoning in east of Iran - a case series. Avic. Jour. of Phytomed. 2(2): 86-89.

Maibam, R. D., Meenakshi, B., Paul, S. B., Sharma, G. D. (2011): Neurotoxic and medicinal properties of *Datura stramonium* L. – Review. Assam University Journal of Science & Technology: Biological and Environmental Sciences. 7(1): 139-144.

Oljača, S., Simić, M., Vrbničanin, S., Stefanović, L., Kovačević, D. (2002): Effect of density and plant position of *Datura stramonium* on maize productivity. Proceed. of the 12th EWRS Symposium, Wageningen, Netherlands, 292-293.

Oseni, O. A., Olarinoye, C. O., Amoo, I. A. (2011): Studies on chemical compositions and functional properties of thorn apple (*Datura stramonium* L.) Solanaceae. African Journal of Food Science. 5(2): 40 – 44.

Richardson, W. H., Slone, C. M., Michels, J. E., Pharm, D. (2007): Herbal drugs of abuse: An emerging problem. Emergence Medicine Clinics of North America. 25: 435–457

Spina, S. P., Taddei, A. (2007): Teenagers with Jimson weed (*Datura stramonium*) poisoning. CJEM. 9:467–8.

Steenkamp, P. A., Harding, N. M., Van Heerden, F. R., Van Wyk, B. E. (2004): Fatal Datura poisoning: identification of atropine and scopolamine by high performance liquid chromatography/photodiode array/mass spectrometry. Forensic Science International. 145(1): 31-39.

Şanlıdağ, B., Derinöz, O., Yıldız, N. (2014): A case of pediatric age anticholinergic intoxication due to accidental *Datura stramonium* ingestion admitting with visual hallucination. The Turkish Journal of Pediatrics 56: 313-315

Šćepanović, M., Novak, N., Barić, K., Ostojić, Z., Galzina, N., Goršić, M. (2007): Alelopatski utjecaj korovnih vrsta *Abutilon theophrasti* Med. i *Datura stramonium* L. na početni razvoj kukuruza. Agronomski glasnik, 6, 459-472.

van Kleunen, M., Fischer, M., Johnson, S. D. (2007): Reproductive assurance through self-fertilization does not vary with population size in the alien invasive plant *Datura stramonium*. Oikos 116: 1400–1412.

Wiebe, T. H., Sigurdson, E. S., Katz, L. Y. (2008): A case report of Angel's trumpet (*Datura stramonium*) poisoning and delirium in adolescents in Winnipeg, Manitoba: Summer 2006. Paediatr Child Health. 13(3): 193-196.

THE IMPACT OF CLIMATE CHANGE ON PLANT PRODUCTION IN SARAJEVO CANTON*

UTICAJ KLIMATSKIH PROMJENA NA BILJNU PROIZVODNJU U KANTONU SARAJEVO

Fejzo Bašić[1], Mirha Điki膹1], Hamid Čustović[1], Nedžad Karić[1], Nedžad Voljevica[2], Sanel Haseljić[1]

Original scientific paper

Summary

In the last decades of the 20th century there has been an increased public concern about the impact of global climate change on human activity, particularly in agriculture as its most sensitive part. This paper shows the expected trends of local warming of the atmosphere for the area of the Sarajevo Canton, and consequently predicts its impacts to the crop production. The trend of examined parameters was estimated using the least squares method. Reducing of the amount of rainfall was calculated from the relationship of potential evapotranspiration and precipitation. KlimaSoft 2.2 program was used for calculation. Phenological stages of certain plant species were thoroughly analysed. An increase in air temperature on the annual and seasonal level was observed. Comparison of the data for the period 1999-2013 with standard climate period (1961-1990) shows an increase in temperature and potential evapotranspiration, along with uneven distribution of precipitation. All above mentioned led to the extension of the growing season for cryophilic and thermophilic species by an average of 14 or 15 days and the rainfall deficite in July and August.

Key words: *climate change, Canton Sarajevo, plant production*

Rezime

U posljednjim decenijama dvadesetog vijeka porasla je zabrinutost javnosti zbog uticaja globalnih klimatskih promjena na ljudsku djelatnost, posebno na poljoprivredu kao njen najosjetljiviji dio. U ovom radu su za područje Kantona Sarajevo predstavljeni očekivani trendovi lokalnog zagrijavanja atmosfere, a shodno tome predviđeni su uticaji istih na biljnu proizvodnju. Procedura analize trenda je procijenjena metodom najmanjih kvadrata. Smanjenje količine padavina se računalo iz odnosa količine padavina i potencijalne evapotranspiracije. Za obračun je korišten

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Faculty of Agriculture and Food Sciences, University of Sarajevo, Bosnia and Herzegovina
[2] Federal Hydrometeorological Institute, Bardakčije 12, 71000 Sarajevo, Bosnia and Herzegovina

program KlimaSoft 2.2. Detaljno su analizirane fenološke faze razvoja pojedinih biljnih vrsta. Osmotreno je povećanje temperature zraka na godišnjem nivou i na nivou svih godišnjih doba. Poređenjem podataka za period 1999-2013 sa standardnim klimatskim periodom, uočeno je povećanje potencijalne evapotranspiracije zajedno sa neravnomjernom raspodjelom količine padavina. Sve zajedno za posljedicu ima produženje perioda vegetacije kriofilnih i termofilnih vrsta u prosjeku za 14, odnosno 15 dana i deficit padavina u julu i avgustu.

Ključne riječi: *klimatske promjene, Kanton Sarajevo, biljna proizvodnja*

INTRODUCTION

Climate change is one of the biggest challenges we face today and its impact is evident around the world. There are several definitions of climate change and most of them indicate that these are the changes in climate that can be assigned to various activities that change the composition of the atmosphere and which are recorded over a longer period of time. The term "climate change" can be used to describe changes in climate that occur as a result of natural and/or human factors. There is still an ongoing discussion on climate change in the scientific world, but according to the 4th report of the Intergovernmental Panel on Climate Change (IPCC, 2007), climate is changing and climate changes are mainly due to human activity. Among the main conclusions of this report, it is noted that the emissions of greenhouse gases resulting from human activities significantly influence the increased warming of the atmosphere.

Over the past 200 years, the atmospheric CO_2 concentration has increased by 31%, while other greenhouse gases and micron-size aerosols follow the same trend and continue to increase. Based on the envisaged increase in concentration of greenhouse gases, climate models predict a global warming of 1.7 to 4.9 °C between 1990 and 2100 (Karl & Trenbeth, 2003).

The average air temperature in Bosnia and Herzegovina has increased by 0.8°C over the past 100 years (which is in line with global trends), with a tendency to increase, because the last decade (2000-2010) was the warmest in the last 120 years. The precipitation amount, depending on the region of Bosnia and Herzegovina, shows minimal changes over the last 100 years (a maximum ± 5%), but it should be noted that there is a trend of increasing precipitation in the central, and decreasing in southwest, north and northeast regions. In general, there are different trends from season to season. In most parts of Bosnia and Herzegovina, there is a negative trend in spring and summer, and increased percipitation amount in winter (UNDP BiH, 2013).

Plant production, as the primary production in agriculture, greatly depends on the climate characteristics of the given area. Due to small investments in the improvement of crop production, the impact of climate change is particularly high in underdeveloped and developing countries, such as Bosnia and Herzegovina. The current CO_2 concentration in the atmosphere is not optimal for plants. They benefit from the CO_2 concentration several times higher than the one present in today's atmosphere, which led to the practice of "fertilization" with carbon dioxide in greenhouses (Šarić & Đikić, 1997). It is anticipated that in the 21st century, cultural plants will produce about 30% more food and fiber due to doubled CO_2 concentration in the atmosphere and increased photosynthesis (Senft, 1990). Increasing temperature by 1-2 degrees might contribute to a significant increase in plant production, and the production area of many crop, fruit and other plants would extend further to the north and to higher altitudes. However, global warming also has negative consequences on agricultural production, such as flooding of areas situated in lower altitudes, higher drought, increased survival of weeds and plant pests, increased number of their generations in a single year and migration of species from warm regions (UNDP BiH, 2013).

The aim of this paper is to provide the expected trends of local atmosphere warming based on the analysis of meteorological parameters for the area of Sarajevo Canton, and accordingly anticipate its impact on plant production.

MATERIAL AND METHODS

The analysis of climate change in an instrumental period is essentially based on the study of temperature and precipitation, since these are the two most important climatic elements. The data on the average air temperature and precipitation used in this paper were obtained from the Bjelave meteorological station, which has a homogeneous series of observations and measurements from 1888 to the present, which is repressive for this analysis.

Data for the period 1888-2013 were used to analyze the fluctuations and trends of mean annual air temperatures and annual precipitation in Sarajevo Canton. One of the most widely used methods for analyzing temperature and precipitation fluctuations is the method of moving or sliding averages (Hadžić & Drešković, 2014). The trend analysis procedure determined by the smallest squares method assists in assessing whether temperatures and precipitation statistically increase or decrease over time.

The trends and differences in air temperature and rainfall to the level of seasons (spring, summer, autumn and winter) were analyzed in detail for the period 1984-2013. and standard climatic period 1961-1990. In order to examine the impact of climate change on the flora, it is appropriate to study the phenophases of the development of those perennial plant species that were not affected by agrotechnical measures. Therefore, all available phenotypic data of lilac (*Syringa vulgaris* L.) and horse chestnut (*Aesculus hippoccastanum* L.) for the reference period (1961-1990) and the five-year series (2009-2013) were analyzed with the aim of detecting deviations. The analyzed phenophases in those species were the beginning of flowering in lilac and beginning of leafing and beginning of flowering in horse chestnut. Based on the average air temperatures, the temperature thresholds of 5 and 10 °C for the period

1961-1990 and 1999-2013 were calculated, and these thresholds indicate the beginning and end of the vegetation of cryophylic and thermophilic plant species (Šarić *et al*., 2010). The reduction of precipitation during the vegetation period was calculated from the relationship between potential evapotranspiration (PET) and precipitation (P). Potential evapotranspiration was determined by Thornthwaite method. KlimaSoft 2.2 was used for the calculation. Meteorological and phenological data were taken from the Federal Hydrometeorological Institute of Bosnia and Herzegovina.

The analysis of climate change in an instrumental period is essentially based on the study of temperature and precipitation, since these are the two most important climatic elements. The data on the average air temperature and precipitation used in this paper were obtained from the Bjelave meteorological station, which has a homogeneous series of observations and measurements from 1888 to the present, which is repressive for this analysis.

Data for the period 1888-2013 were used to analyze the fluctuations and trends of mean annual air temperatures and annual precipitation in Sarajevo Canton. One of the most widely used methods for analyzing temperature and precipitation fluctuations is the method of moving or sliding averages (Hadžić & Drešković, 2014). The trend analysis procedure determined by the smallest squares method assists in assessing whether temperatures and precipitation statistically increase or decrease over time.

The trends and differences in air temperature and rainfall to the level of seasons (spring, summer, autumn and winter) were analyzed in detail for the period 1984-2013. and standard climatic period 1961-1990. In order to examine the impact of climate change on the flora, it is appropriate to study the phenophases of the development of those perennial plant species that were not affected by agrotechnical measures. Therefore, all available phenotypic data of lilac (*Syringa vulgaris* L.) and horse chestnut (*Aesculus hippoccastanum* L.) for the reference period (1961-1990) and the five-year series (2009-2013) were analyzed with the aim of detecting deviations. The analyzed phenophases in those species were the beginning of flowering in lilac and beginning of leafing and beginning of flowering in horse chestnut. Based on the average air temperatures, the temperature thresholds of 5 and 10 °C for the period 1961-1990 and 1999-2013 were calculated, and these thresholds indicate the beginning and end of the vegetation of cryophylic and thermophilic plant species (Šarić *et al*., 2010). The reduction of precipitation during the vegetation period was calculated from the relationship between potential evapotranspiration (PET) and precipitation (P). Potential evapotranspiration was determined by Thornthwaite method. KlimaSoft 2.2 was used for the calculation. Meteorological and phenological data were taken from the Federal Hydrometeorological Institute of Bosnia and Herzegovina.

RESULTS AND DISCUSSION

Changes in air temperature on annual basis

The mean annual air temperature of the entire instrumental period (1888-2013) was 9.64°C. The concrete value is determined by the linear function y = ax + b, where the quantitative values of the parameters of the equation a and b are determined by the method of the sum of the smallest squares, which for the mean annual temperatures have the following values: a = 0.0095 and b = 8.7773. In this way, the trend in the movement of average annual temperatures is determined with the value of warming in the entire instrument period of 0.95 °C (Figure 1). At the beginning of this millennium the trend was 0.6 °C (Majstorović, 2001). The analysis of five-year moving averages revealed that the last period, which lasts from 1999 to 2013, is characterized by the most abundant rise in the average temperature of 10.6 °C, which can be labeled as the warmest period in the overall instrumental observation.

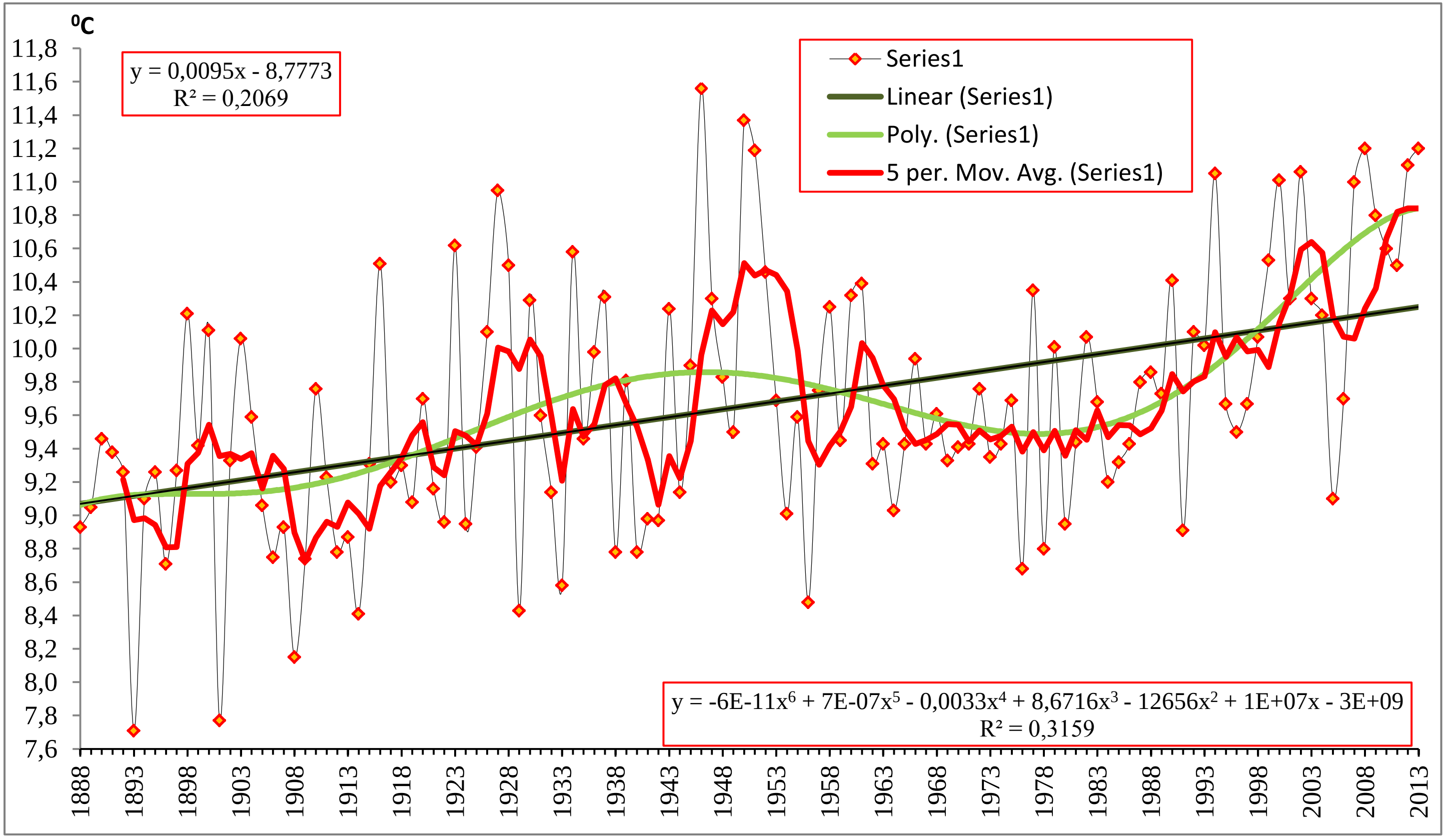


Figure 1. The flow of mean annual temperature with climate fluctuation parameters

Changes in air temperature at the seasonal level

Based on the comparative analysis for the 1984-2013 and 1961-1990 periods, a positive trend in the movement of mean air temperatures to the level of all seasons has been determined. A positive trend with the highest warming value (1.16 °C) was detected during summer, and positive trend with the lowest warming value (0.3 °C) during winter. During spring and autumn there has been an increase of 0.4 °C.

Precipitation changes on annual basis

The average value of annual precipitation in the period from 1888 to 2013 was 923 mm. The analysis of linear trend indicates that the annual rainfall in the instrumental period increased to about 33 mm (Figure 2), meaning that there is a barely noticeable humidification of the climate inside Sarajevo (Hadžić & Drešković, 2014). The analysis of five-year sliding centers can determine the existence of several rounded cycles within which all existing precipitation tendencies are expressed.

Seasonal precipitation changes

Based on the comparative analysis for the 1984-2013 and 1961-1990 periods, there are different trends from season to season. The greatest decrease in precipitation is during the summer (-17 mm), and the greatest increase in precipitation was observed in autumn (+28 mm). The increase of precipitation trend, to a much lesser extent (of only a few mm), was registered during spring and winter.

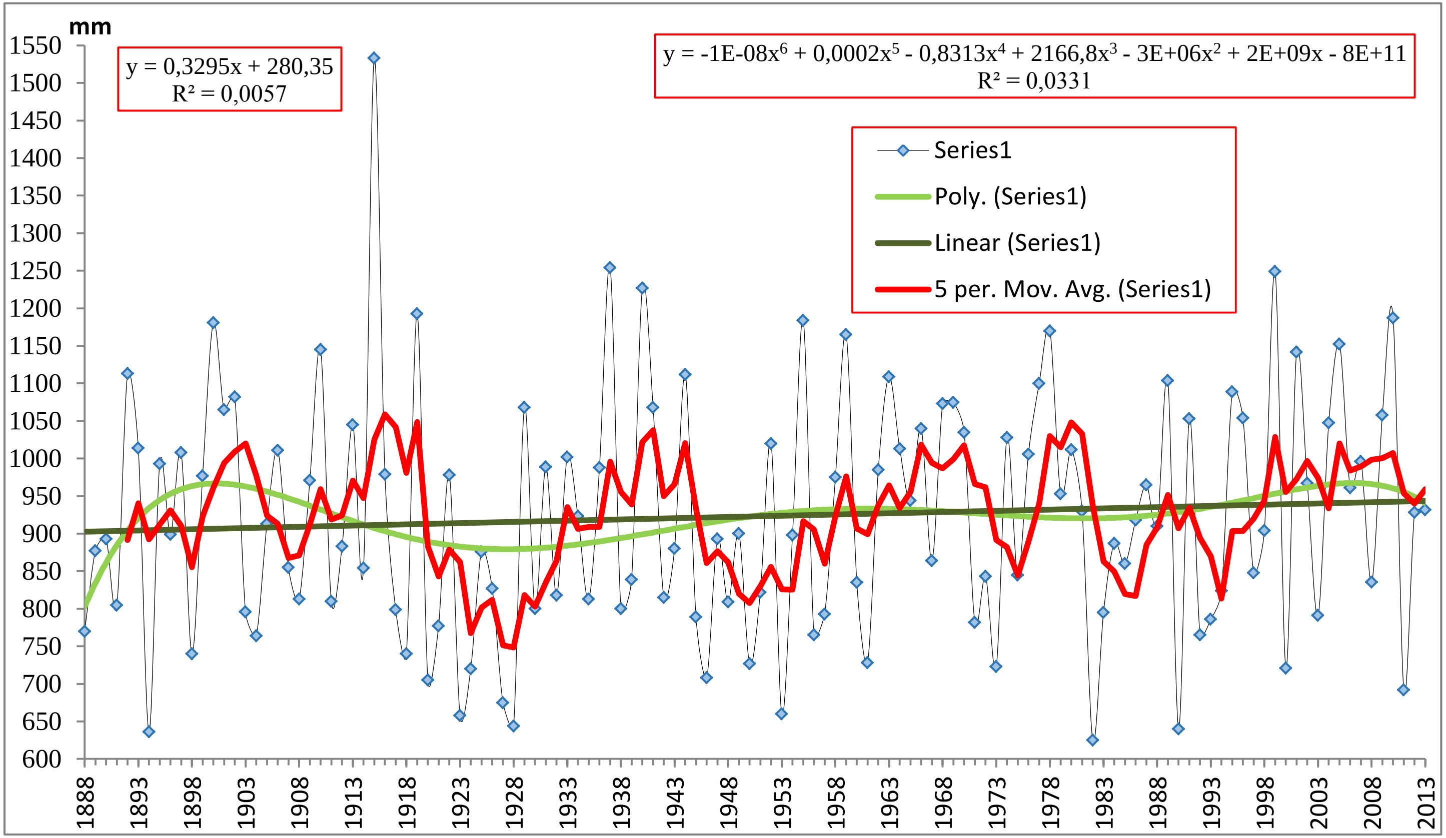


Figure 2. The flow of annual precipitation with climate fluctuation parameters

The influence of air temperature on the phenophases and the length of vegetation period

The comparison of phenological data for the period 2009-2013 with the reference period showed that beginning of flowering in lilac occured three days earlier. In horse chestnut, leafing occured one day, and flowering three days earlier. There is a number of studies that emphasize the effect of climate change on the phenophases of leafing and flowering of plants (Menzel, 2003; Cleland *et al*., 2007). On the basis of the comparison of the phenophases of lilac, Jelić & Vučetić (2012) have observed the earlier beginning of leafing, flowering and full flowering for 2-8 days in 10 years over the last five decades. In NE America, lilac development phenophases occur in 2-8 days/10 years earlier (Wolfe *et al*., 2005). The analysis of the linear trends of the phenological phases of different apple varieties for selected stations in Croatia showed a significantly earlier starting of leafing and blooming (Krulić & Vučetić, 2012). Changes in the onset of initial development phases of plants are a consequence of increased insolation in spring and winter, which causes an increase in mean winter minimum and spring maximum air temperatures (Vučetić & Vučetić, 2006).

From 1961. to 1990. a period with average temperatures above 5 °C, or the period of active vegetation of cryophilic plant species in Sarajevo was from March 15 to November 17, meaning that it lasted 243 days on average. From 1999 to 2013, the period with average temperatures above 5 °C was from March 11 to November 28, and lasted 257 days on average. The period with average temperatures above 10 °C, or the period of active vegetation of thermophilic plants from 1961 to 1990 was from April 19 to October 17, which means that it lasted 181 days on average. From 1999-2013, the period with average temperatures above 10 °C was from April 10 to October 23, and lasted 196 days on average. From the above examples, it can be noticed that the period of active vegetation of both cryophilic and thermophilic plants begins several days earlier in the spring and ends several days later in autumn. As a result, the active vegetation of cryophilic plants is longer on average for 14 days, and thermophilic for 15 days compared to the standard climatic period.

Coefficient of precipitation reduction (P/PET)

The ratio of precipitation (P) and potential evapotranspiration (PET) is calculated as a measure of precipitation reduction. Based on this relationship, the amount of evapotranspiration required is compensated for precipitation for the period of vegetation (April-September). Long-term data series were analyzed in the periods 1961-1990 and 1999-2013. According to the criteria adopted by the UNEP (2011), when the P/PET ratio is below a certain limit of 0.05>P/PET/<0.65, the given area is susceptible to converting into the desert, and when the ratio is above this limit, there is no possibility of converting to a dry zone. In table 1, the dry periods are marked with red (0.05<P/PET<0.20), the half-dry periods with orange (0.20<P/PET <0.50), and sub-humid periods with yellow (0.50<P/PET<0.65). In the period 1999-2013, an increase in the number of drought months and a decrease in drought coefficient can also be observed even in months which were not marked by color. Although the deficit can occur in all months of the vegetation period, its highest values appear in July and August, when most plants have the the greatest need for water. By comparing the data for the period 1999-2013, with the standard climatic period 1961-1990, the temperature and potential evapotranspiration (PET) increased, along with the uneven distribution of precipitation during the vegetation period, which resulted in a decrease in water supplies. Based on the deviation, the entire period of vegetation shows the decreasing trend in percipitation amount, ie the trend of increasing drought periods. Those results are confirmed by the results of Hodžić *et al*. (2013), who conducted a study in the 2001-2012. period and concluded that there was a decrease in the coefficient of the amount of precipitation or the more frequent occurrence of drought periods compared to the standard climatic period in Sarajevo Canton.

Tab. 1. Reduction of precipitation (P / PET) during the vegetation period

	IV	V	VI	VII	VIII	IX
1999	1,90	0,69	0,65	0,81	0,71	1,1
2000	0,84	0,80	0,19	0,40	0,12	0,89
2001	2,54	0,77	1,15	0,49	0,56	3,65
2002	2,36	0,89	0,41	0,58	0,81	3
2003	0,70	0,46	0,72	0,47	0,06	1,09
2004	2,05	1,29	0,71	1,06	0,34	0,89
2005	1,40	1,22	0,73	0,85	1,34	1,36
2006	1,37	0,66	0,73	0,91	1,63	0,53
2007	0,31	1,1	0,45	0,46	0,18	2,24
2008	1,12	0,59	0,68	0,76	0,33	1,23
2009	0,95	0,63	1,36	0,64	0,43	0,24
2010	1,09	1,05	1,57	0,21	0,34	1,7
2011	0,57	1,21	0,63	1	0,04	0,4
2012	2,18	1,73	0,08	0,24	0,2	0,98
2013	1,07	1,57	0,57	0,21	0,30	1
average	1,36	0,97	0,7	0,6	0,49	1,35
1961-1990	1,5	1,01	0,83	0,7	0,54	1,36
deviation	**-0,14**	**-0,04**	**-0,13**	**-0,1**	**-0,05**	**-0,01**

CONCLUSION

Changes in air temperature and precipitation, with the expected increase in CO_2 concentration, will have different effects on plant production in Canton Sarajevo. The positive impact of climate change is reflected in the extension of the vegetation period, which will begin earlier in the spring and end later in autumn. Further increase in air temperature, with the increase in CO_2, will result in an increase in the photosynthesis and yield of a large number of plants. The growing area of plants at higher altitudes will increase. It is expected to reduce the freezing of all those species that are sown and planted in autumn. Of all unfavorable weather conditions, the increasing deficit of precipitation in the warmer part of the year and flooding of lower areas in some years will have the most devastating impact on plant production in the Sarajevo Canton.

REFERENCES

Cleland, E., Chuine, I., Menzel, A., Mooney, H., Schwartz, M. (2007): Shifting plant phenology in response to global change. Trends in Ecology and Evolution, 22: 357-365.

Hadžić, E., Drešković, N. (2014): Analiza uticaja temperaturnih i padavinskih oscilacija na riječne protoke u Sarajevskoj kotlini. Vodoprivreda, 46:65-75.

Hodžić, S., Majstorović, Ž., Bijedić, A. (2013): Global climate changes on the territory of Bosnia and Herzegovina on their impact on agriculture. Proceedings of the 24th International Scientific-Expert-Conference of Agriculture and Food Industry, Sarajevo, Bosnia and Herzegovina, pp. 26-32.

IPCC (2007): Climate Change 2007a: Synthesis Report Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Geneva, 104 pp.

Jelić, M., Vučetić, V. (2012): Utječe li promjena klime na početak cvjetanja jorgovana? Hrvatski meteorološki časopis 46:45-43.

Karl, T. R., Trenbeth, K. E. (2003): Modern global climate change. Science. 302:1719–1723.

Krulić, B., Vučetić, V. (2012): Razvojne faze i zimsko mirovanje jabuke u Hrvatskoj. Hrvatski meteorološki časopis 46:35-43.

Majstorović, Ž. (2001): Klimatološke procjene u svjetlu izvještaja za Sarajevo za period od 100 godina. Okrugli sto: Okvirna konvencija UN za klimatske promjene (UN FCCC) i Kjoto protokol, Zbornik radova, Banja Luka.

Menzel, A. (2003): Plant phenological anomalies in Germany and their relation to air temperature and NAO. Climatic Change, 57:243-263.

Senft, D. (1990): Greenhouse Effect May Not Be All Bad. Agric Research, Vol. 38, No. 10: 20-23, Washington, D. C.

Šarić, T., Đikić, M., (1997): Promjene klime pod uticajem čovjekovih aktivnosti i uticaj tih promjena na biljnu proizvodnju. Radovi Poljoprivrednog fakulteta Univerziteta u Sarajevu, God. XLII, broj 46/1997.

Šarić, T., Đalović, I., Đikić, M. (2010): Opšte ratarstvo-praktikum. Poljoprivredni fakultet Univerziteta u Sarajevu.

UNDP BiH (2013): Drugi nacionalni izvještaj Bosne i Hercegovine u skladu sa Okvirnom konvencijom Ujedinjenih nacija o klimatskim promjenama.

UNEP (2011): Keeping Track Our Changing Environment: From Rio to Rio + 20 (1992-2012), ISBN: 978-92-802.

Vučetić, V., Vučetić, M. (2006): Phenological fluctuations as a possible signal of climatic changes in the Croatian mounatain area. Meteorogische Zeitschrift, 15 (2):237-242.

Wolfe, D. W., Scwartz, A., Lakso, N. (2005): Climate change and shifts in spring phenology of tree horticultural woody perennials in northeastern USA. International Journal of Biometeorology, 49: 303-309.

PROCJENA EROZIJE I MOGUĆE MJERE KONZERVACIJE POLJOPRIVREDNOG TLA NA PODRUČJU ŽELJEZNOG POLJA

ASSESSMENT OF EROSION AND POSSIBLE CONSERVATION MEASURES OF AGRICULTURAL SOIL IN THE AREA OF ŽELJEZNO POLJE

Jasminka Žurovec[1], Sabrija Čadro[1], Kenan Sinanović[2], Samir Husić[1], Dženita Šehić[1], Ajla Mrkulić[1]

Original scientific paper

Rezime

Erozija tla vodom je jedan od najznačajnijih uzroka degradacije tla u Bosni i Hercegovini (BiH). Posebno je prisutna na malim farmama koje se često nalaze u rubnim područjima gdje je kvalitet tla loš, a topografija vrlo izražena, kao što je to slučaj i sa Željeznim Poljem na kome su vršena istraživanja predstavljena u ovom radu.

Područje Željeznog Polja koje se nalazi u blizini Žepča u centar pažnje javnosti dolazi u maju 2014. godine kao jedno od najviše stradalih područja od katastrofalne erozije tla i poplava koje su te godine zadesile BiH. Kako životna egzistencija stanovnika ovog područja skoro u potpunosti ovisi o poljoprivredi, danas se erozijom izgubljeni fond obradivog zemljišta nastoji nadoknaditi preoravanjem pašnjaka, a nerijetko i krčenjem šuma, što u budućnosti može prouzročiti nove okolišne probleme.

U ovom radu, za procjenu erozije korištena je Univerzalna jednačina gubitka tla erozijom – USLE. U skladu s procijenjenim vrijednostima potencijalne erozije, stvarne erozije i rizika od erozije, date su odgovarajuće preporuke vezane za način korištenja tla, koje bi omogućile da se količina erozionog nanosa na ovom području svede na tolerantni nivo i drži pod kontrolom.

Ključne riječi: *USLE, stvarna erozija, rizik od erozije, konzervacija tla*

Summary

Soil water erosion is one of the most important causes of soil degradation in Bosnia and Herzegovina (BiH), this is especially true for smallholder farms that are often located in marginal areas, where the soil quality is poor and the topography is complexed. At one such location, called Željezno Polje, research presented in this paper was carried out.

[1] Poljoprivredno-prehrambeni Fakultet Univerziteta u Sarajevu – Faculty of Agriculture and Food Sciences, University of Sarajevo
[2] Poljoprivredna zadruga "Poljar", Žepče

Željezno Polje in May 2014 becomes interesting to the public, this area was exposed to significant damage caused by erosion and flooding. As the people in this area almost completely depend on the agriculture production, lost arable lands are compensated by plowing pastures and deforestation. Such practices can cause serious problems in the near future.
In this study, in order to assess the erosion - universal soil loss equation (USLE) was used. In accordance with the obtained potential erosion, actual erosion and risk of the erosion values, this work provides the appropriate recommendations regarding the method of land use. By applying this method, smallholeder farmers at Željezno Polje can reduce and keep under control the amount of erosion deposits.

Key words: *USLE, actual erosion, risk of erosion, soil conservation*

UVOD

Brzi rast stanovništva i povećani zahtijevi u pogledu korištenja postojećih prirodnih resursa, među kojima tlo zauzima posebno mjesto, doveli su do ubrzane degradacije okoliša i smanjenja produktivnost svih prirodnih ekosistema.
U 21. stoljeću posebno je aktuelan problem degradacije tla, zbog velikog uticaja koji ima na poljoprivredu, okoliš i sigurnost hrane (Eswaran, i sar., 2001). Procjenjuje se da je do 40% poljoprivrednog zemljišta u svijetu teže degradirano (Sample, 2007). Obzirom da se više od 99,7 % hrane proizvede na zemlji, a manje od 0,3 % dolazi iz mora i drugih vodnih ekosistema (FAO, 2013), mogućnost održavanja i povećavanja njene proizvodnje u budućnosti će prvenstveno zavisiti od produktivnosti i kvaliteta postojećeg fonda poljoprivrednog zemljišta.
Najveći značaj u globalnom procesu degradacije tla ima erozija. Ona je ujedno i jedan od najozbiljnijih ekoloških problema povezanih s načinom korištenja tla (Morgan, 1996). Erozija je složen proces koji zavisi od svojstva tla, reljefa, vegetacije, količine i intenziteta padavina (Selby, 1993). U tom procesu se tlo pod uticajem vode ili vjetra premješta sa jedne lokacije na drugu i najčešće nepovratno gubi. Najugroženiji je površinski sloj, najbogatiji organskom materijom i hranjivima, koji je kao takav i najprikladniji za uzgoj biljaka.

Erozija izazvana antropogenim uticajem i posljedice njenog djelovanja tokom niza godina doveli su do napuštanja ili smanjenja produktivnosti vrijednog fonda poljoprivrednog zemljišta, u koji su do tada uložena značajna sredstava (Pimentel, i sar., 1995; Young, 1998; Pimentel, 2006). Svake godine erozijom se izgubi oko 10 miliona hektara obradivog zemljišta. U kopnenim područjima tlo se gubi 10 do 40 puta brže nego što se obrazuje, što u budućnosti može predstavljati ozbiljnu prijetnju za sigurnost proizvodnje hrane i kvalitet okoliša (Pimentel, 2006). Gubitak obradivih površina djelovanjem erozije često rezultira stvaranjem novih na uštrb šumskog zemljišta i pašnjaka uz potrebu njihovog dodatnog obogaćivanja azotnim i fosfornim đubrivima (Pimentel, 2013).

Promjene u načinu korištenja zemljišta mogu znatno da ubrzaju proces erozije tla (Pimentel, 1987; Ursic, i sar., 1965; Wolman, 1967; Hooke, 2000), a odavno je poznato da će erozija koja premašuje stopu obnavljanja tla za posljedicu imati smanjenje poljoprivrednih potencijala (Shaler, 1905; Lowdermilk, 1953; Dale i sar., 1955; Brink, i sar., 1977; Pimentel, i sar., 1987).
U literaturi se navode mnoge rasprave o mogućim pragovima iznad kojih eroziju tla treba posmatrati kao ozbiljan problem. U sklopu njih uveden je pojam "tolerantna" erozija - *T* ($t\ ha^{-1}\ god^{-1}$) koja bi se trebala odrediti na bazi pouzdanih procjena prirodne stope obnavljanja (formiranja) tla. Međutim, stope i procesi formiranja tla se znatno razlikuju. To je razumljivo uzme li se u obzir da se proces nastanka 1 cm tla, obzirom na konstelaciju pedogenetskih faktora, može odvijati od 1 000 do 10 000 godina (Resulović, i sar. 2008). U nekim slučajevima, erozije tla veća od 1 tone po hektaru godišnje ($t\ ha^{-1}\ god^{-1}$) se smatra podnošljivom iz šire perspektive društva kao cjeline, na primjer za ekonomska razmatranja ili očuvanje funkcije tla. U Švicarskoj npr., prag do koga se erozija toleriše je obično $1\ t\ ha^{-1}\ god^{-1}$, iako se ona za neke tipove tla može povećati i na $2\ t\ ha^{-1}\ god^{-1}$ (Schaub, i sar., 1998). Odskora, Verheijen i sar. (2009) procijenjuju da u Evropi prosječna stopa formiranja tla iznosi $1,4\ t\ ha^{-1}\ god^{-1}$ što je znatno niže od procijenjene stope gubitka tla. Iako se gubici tla iznad $1\ t\ ha^{-1}\ god^{-1}$ smatraju kao nepovratni, ipak, postoji potreba za predlaganjem različitih vrijednosti tolerantne erozije (*T*) koje bi bile prihvatljive, za različita tla, u različitim dijelovima Svijeta.

U BiH se o eroziji tla na poljoprivrednim površinama vrlo malo govori. Pažnja javnosti se na taj problem najčešće usmjeri tek onda kada je već kasno da se nešto poduzme, kada se već pričini velika materijalna šteta ili nepovratno izgubi plodno tlo tamo gdje ga ionako nema dovoljno, ali i tada, na žalost, više u kontekstu njegovih indirektnih posljedica. Tako je bilo i sa Željeznim Poljem u blizini Žepča (centralna Bosna) u maju 2014. godine, koje je tada postalo poznato kao jedno od najviše stradalih područja od erozije i katastrofalnih poplava koje su te godine zadesile BiH. Iako su i danas svuda uokolo vidne posljedice djelovanja bujičnih tokova, klizišta i erozije uopšte, njegovi stanovnici ne napuštaju ovaj kraj i nastavljaju se baviti poljoprivrednom proizvodnjom od koje uglavnom žive. Postojeći agroekološki uslovi i usitnjene parcele opredijelili su ih za proizvodnju jagodičastog voća, koja je u ekspanziji. Najčešće se uzgaja malina koja je zastupljena na većini obradivih površina. Kako zbog položaja parcela i nagiba na kojim se nalaze, te načina na koji se obrađuju i koriste, posebnu pažnju na ovom području treba posvetiti eroziji, osnovni cilj ovoga rada je da se metodom *USLE* (*Universal Soil Loss Equation*) Wischmeiera i Smitha (1978) procijene potencijalna erozija (*RKLS*), stvarna erozija (*A*) i rizik od erozije.
USLE je u svijetu vrlo često korištena metoda za procjenu površinske i brazdaste vodne erozije na poljoprivrednim površinama. To je ujedno i jedina metoda koja je do sada korištena za procjenu erozije na poljoprivrednim površinama u BiH (Žurovec i Čadro, 2008). Iako ima svojih nedostataka i ograničenja, naširoko se koristi zbog

svoje relativne jednostavnosti i dosljednosti (Desmet i Govers, 1996). Kako se *USLE* osim za procjenu erozije može koristiti i u konzervacionoj praksi (zaštiti tla od erozije), u ovom radu korištena je i za sugerisanje određenih aktivnosti koje bi mogle pomoći da se količina erozionog nanosa na ovom području drži pod kontrolom.

MATERIJAL I METODE RADA

Istraživano područje

Željezno Polje, koje se nalazi u blizini Žepča, jedna je od najvećih mjesnih zajednica u BiH. Ukupna površina mu iznosi 4 050 ha, od čega 70 % (2 835 ha) površine čini šumsko zemljište, a svega 1 215 ha poljoprivredno (SROŽ, 2011).
Većina stanovnika ovog kraja tradicionalno se bavi poljoprivredom, čemu pogoduje blizina tržišta većih gradova poput Zenice i Žepča. U posljednje vrijeme, zahvaljujući proizvodnji maline sa kojom se započelo 2001. godine, poljoprivredna proizvodnja na ovom području je poprimila sasvim novi karakter. Na obradivim površinama, svuda unaokolo, podižu se brojni malinjaci koji su postali glavni izvor prihoda stanovnika ovog kraja. Po uzgoju malina Željezno polje postalo je poznato ne samo širom BiH nego i u inostranstvu.
Za potpuniji uvid u pedološke i geološke uslove na ovom području, osim vlastitih istraživanja, u radu su korišteni i podaci iz Pedološke karte BiH u mjerilu 1:50.000 sa tumačem (sekcija Zenica *1*) i Osnovne geološke karte BIH u mjerilu 1:100.000 (sekcija Teslić *L33-132*), a za analizu klimatskih prilika korišteni su dostupni podaci najbliže meteorološke stanice Zenica za period 1961. – 2015. godina, dobivenih od Federalnog hidrometeorološkog zavoda, Sarajevo.
Istraživanja koja su provedena u decembru 2016. godine na području Željeznog Polja podrazumijevala su: terenski rad, laboratorijske analize i obradu podataka.

Terenska istraživanja

Nakon obilaska terena, za potrebe analize hemijskih i fizičkih svojstava i teksture površinskog sloja tla, koji je izložen i zbog toga i najugroženiji erozionim procesima, na 8 reprezentativnih parcela (Tabela 1) uzeti su prosječni uzorci tla do dubine od 15 cm. Na ovim parcelama obavljeno je i mjerenje njihove dužne (m) i prosječnog pada (%). Obavljeno je i mjerenje dužine (m) i prosječnog pada (%) izabranih parcela. Na terenu su također prikupljene informacije o načinu korištenja poljoprivrednog zemljišta, vegetaciji i drugim pokazateljima od značaja za razmatranje i analizu problema erozije na ovom području.

Laboratorijska istraživanja

Laboratorijska istraživanja prosječnih uzoraka tla obavljena su u laboratoriji Instituta za pedologiju agrohemiju i melioracije (*PAM*) Poljoprivredno-prehrambenog fakulteta u Sarajevu. Analizirani su:

- Sadržaj humusa, metodom po Kocmanu
- Struktura tla suhim prosijavanjem, po Savinovu.
- Stabilnost strukture tla mokrim prosijavanjem po Savinovu

- Tekstura tla, hidrometrijskom metodom, uz klasifikaciju teksturnih elemenata prema USDA: glina < 2 µm, prah 2 – 50 µm i pijesak 50 – 2000 µm.
- Sadržaj čestica vrlo finog pijeska (50 – 100 µm) je određen suhim prosijavanjem
- Vodopropusnost, indirektno, na bazi poznavanja teksturnog sastava.

Tabela 1. Lokacije osam reprezentativnih parcela
Locations of eight representative parcels

Lokacija *Location*		Geografska širina *Latitude*	Geografska dužina *Longitude*	Nadmorska visina *Altitude* (m)	Površina *Area* (m^2)	Dužina parcele *Parcel length* (m)	Pad *Slope* (%)
1	Ozimine	44°24'02"	17°55'45"	729	2170	26	36.0
2		44°24'00"	17°55'47"	679	2372	36	32.4
3	Jezerac	44°24'16"	17°55'38"	665	1152	58	14.0
4	Strana	44°24'12"	17°55'33"	670	1659	35	33.3
5	Padež	44°24'11"	17°55'10"	801	2174	21	42.1
6	Jastrebac	44°24'20"	17°54'45"	827	5354	46	34.9
7		44°24'23"	17°54'51"	799	768	41	27.5
8	Samari	44°24'53"	17°54'55"	717	3791	38	25.0

Procjena erozije tla

Za procjenu erozije tla korištena je Univerzalna jednačina gubitka tla erozijom (*Universal Soil Loss Equation – USLE*) Wischmeiera i Smitha (1978):

$$A = R \times K \times LS \times C \times P \qquad \text{(Formula 1)}$$

gdje je: *A* prosječni godišnji gubitak tla erozijom (t ha^{-1} god^{-1}), *R* faktor erozivnosti kiše, *K* faktor erodibilnosti tla, *L* faktor dužine padine, *S* faktor nagiba padine, *C* faktor biljnog pokrova i *P* faktor primijenjenih konzervacionih mjera.

Erozivnost kiše (faktor *R*), koja se sagledava kroz uticaj kišnih kapi na proces erozije, je u nedostatku dnevnih podataka o intenzitetu kiše koje zahtijeva originalna *USLE* metoda, izračunata pomoću Fournierovog indeksa (1960), koji je Arnoldus (1977a, 1980) modifikovao i FAO poslije provjere prihvatio za standardno korištenje, prema kome je:

$$R = \sum_{i=1}^{12} (p_i^2 / P) \qquad \text{(Formula 2)}$$

gdje je: *p* srednja mjesečna količina padavina u promatranom razdoblju u mm,
P godišnja količina padavina u mm.

Erodibilnost tla (faktor K) zavisi od osobina tla, odnosno teksture, sadržaja organske materije, strukture i propusnosti tla. U ovom radu određena je računskim putem (Wischmeier i Smith, 1978; Renard, i sar., 1997), na osnovu Formule 3:

$$K = \left[\left(2{,}1 \times 10^{-4} \times M^{1{,}14}(12 - OM) + 3{,}25(s - 2) + 2{,}5(p - 3)\right)/100\right] \times 0{,}1317$$

gdje je: *OM* organska materija (%); *s* strukturna klasa (*s* = 1: vrlo sitno mrvičasta, *s* = 2: sitno mrvičasta, *s* = 3, srednje ili krupno mrvičasta, *s* = 4: grudve ili blokovi); *p* propusnost (*p* = 1:

velika, p = 2: osrednja do velika, p = 3: osrednja, p = 4: mala do osrednja, p = 5: mala, p = 6: vrlo mala); M faktor teksture, koji se određuje po formuli:

$$M = (m_{silt} + m_{vfs}) \times (100 - mc) \qquad \text{(Formula 4)}$$

gdje je: m_{silt} sadržaj praha (2 – 50 μm), m_{vfs} sadržaj vrlo finog pijeska (50 – 100 μm), a mc sadržaj gline (< 2 μm).

Dužina padine (faktor L) i pad terena (faktor S), su za svaku analiziranu parcelu utvrđeni geodetskim mjerenjima na terenu. Dobivene vrijednosti su pretvorene u koeficijente, korištenjem formule (Robert i Stone, 2012):

$$LS = [0{,}065 + 0{,}0456(S) + 0{,}006541(S)^2](\frac{L}{22{,}1})^{NN} \qquad \text{(Formula 5)}$$

gdje je: NN zavisno do pada (S) iznosi; 0,2 $S < 1$ (%), 0,3 $S \leq 1 < 3$ (%), 0,4 $S \leq 3 < 5$ (%), 0,5 $S \geq 5$ (%).

Biljni pokrov (faktor C), čija se vrijednost kreće od 0 (kada je tlo u potpunosti prekriveno biljkama) do 1 (kada biljnog pokrova na tlu nema), određen je na osnovu dominantno zastupljene biljne kulture na površinama sa kojih su uzeti uzorci tla. S obzirom na biljno-uzgojne zahvate u zasadima maline u Željeznom polju, korištene su vrijednost faktora C = 0,27 (Merritt, i sar., 2001) za malinu i C = 0,08 (USDA, 2008; Kelvin i sar., 2013) u slučaju da je međuredni prostor u zasadu maline cijele godine pod travnim pokrivačem.

Konzervacione mjere (faktor P), ako su primijenjene na poljoprivrednim površinama, mogu znatno uticati na smanjenje erozije na nekom prostoru. Međutim, ako to nije slučaj, kao na području Željeznog Polja, gdje se obrada vrši uz i niz nagib terena, ovaj faktor ima vrijednost 1. U slučaju da se vrši konturna obrada, vrijednost faktora P iznosi 0,5 (Kelvin i sar., 2013).

Samo u slučaju kada je izračunata vrijednost A manja ili jednaka vrijednosti tolerantne erozije (T), smatra se da mjere zaštite tla od erozije nije neophodno provoditi.

Pojam "tolerantna" erozija (T) predstavlja prirodnu - prihvatljivu količinu tla koja se godišnje gubi erozijom. Prema američkim iskustvima, vrijednosti tolerantne erozije se u zavisnosti od dubine tla i matičnog supstrata kreću od 2,2 – 11,2 t ha^{-1} god^{-1} (Arnoldus, 1977b). U skladu s njima, tolerantna erozija je na istraživanom području procijenjena na 4 t ha^{-1} god^{-1}.

Nakon što su određene vrijednosti pokazatelja *R, K, LS, C, P* i vrijednost tolerantne erozije (*T*) za područje Željeznog polja, njihovom međusobnim kombinovanjem, izračunate su:

- Potencijalna erozija (*RKLS*), koja predstavlja prosječan gubitak tla erozijom (t ha^{-1} god^{-1}) koji bi se, obzirom na osobine padavina i tla, na određenom nagibu pojavio na golom, nezasijanom tlu oranom u smjeru nagiba terena (uzbrdo i nizbrdo);
- Stepen rizika od erozije ((*RKLS/T) x 100*), koji ukazuje na potrebu poduzimanja protiverozionih zahvata, u datim okolnostima;

- Stvarna erozija (*RKLSCP*), koja predstavlja prosječan gubitak tla erozijom (t ha^{-1} god^{-1}) koji se može očekivati pri uzgoju neke kulture, odnosno u praktikovanom plodoredu, praktikovanoj obradi, i eventualno drugim pratećim biljno-uzgojnim ili konzervacionim zahvatima.

Za ocjenu stepena rizika od erozije korišten je kriterij koji predlažu Auerswald i Schmidt (1986).

REZULTATI I DISKUSIJA

Željezno Polje se nalazi na obroncima planine Mahnjače (1360 m.n.v.). Reljef ovog područja je pretežno brdsko-planinski, vrlo je izlomljen i ispresijecan brojnim stalnim i povremenim vodotocima. Većina naseljenih mjesta i pripadajućeg obradivog zemljišta nalazi se na 600 – 700 m.n.v. Reljef okolnog prostora, koji obezbjeđuje zaklonjenost i prostornu izolovanost Željeznog Polja od ostalog prostora, značajno utiče na njegov mikroklimat.

Oko 70% površina ovog područja zauzimaju šume bukve, hrasta, smrče i bora. Ostale površine, koje se uglavnom koriste u poljoprivredi, su pašnjaci ili obradivo zemljište. Na obradivim površinama upadno dominiraju zasadi jagodičastog voća, prvenstveno maline, po kojima je posljednjih godina ovaj kraj postao prepoznatljiv.

Prema podacima Osnovne geološke karte 1:100.000, (sekcija Teslić L33-132) područje Željeznog polja je geološko-litološki uglavnom građeno od Jurskih neraščlanjenih vulkanogeno-sedimentnih stijena, kao što su pješčari, glinci, laporci, rožnjaci sa malo dijabaza i spilita. Površinskim raspadanjem ovih stijena nastaje veća količina trošine (detritusa) različitih dimenzija koja je jako podložna transportu silom gravitacije.

Na navedenom geološkom supstratu pretežno su se razvila kisela smeđa plitka i srednje duboka, skeletoidna, vrlo jako do jako kisela tla (pH od 4,7 do 5,3). U površinskom horizontu, koji je najugroženiji erozionim procesima, su dosta humozna. Po teksturnom sastavu su praškaste ilovače do ilovače, skeletoidne. Pretežno imaju sitno mrvičastu, nestabilnu, strukturu, koju odlikuje visok stepen raspadanja. Sadrže dosta sitnijeg i krupnijeg skeleta, koji im omogućava osrednju propusnost (Tabela 2).

Tabela 2. Hemijske i fizičke karakteristike tla sa osam analiziranih lokacija
Soil chemical and physical characteristics

Lok.	*pH*		Humus	Str*	Pro**	Glina *Clay*	Prah *Silt*	Pijesak *Sand*	Skelet *Skeleton*	Teksturna klasa USDA
	H_2O	KCl				< 0.002	0.002 - 0.05	0.05 - 2	> 2	
			%			%	%	%	%	
1	5,2	4,4	4,81	3	3	9	43	48	17	I
2	4,7	3,9	3,82	3	3	10	47	43	20	I
3	5,0	4,1	3,71	3	3	10	53	37	14	PrI
4	5,2	4,4	3,01	3	2	6	38	56	42	PjI
5	4,7	3,9	4,73	3	3	8	53	39	25	PrI
6	5,3	4,3	3,74	3	3	8	52	40	16	PrI

7	5,2	4,3	3,77	3	3	9	64	27	20	PrI
8	5,2	4,1	3,08	3	3	12	70	18	19	PrI

*Struktura: 3 – Srednje do krupno mrvičasta; **Propusnost: 2 – osrednja, 3 – osrednja do velika; ***I – Ilovača, PrI – Praškasta ilovača, PjI – Pjeskovita ilovača

Kao što je u metodici rada navedeno, za procjenu erozije tla na ovom području korištena je Univerzalna jednačina gubitka tla erozijom (*Universal Soil Loss Equation – USLE*) Wischmeiera i Smitha (1978).

Na osnovu mjesečnih podataka o padavinama (period 1961. – 2015. godina), izračunata je (Formula 2) vrijednost erozivnosti kiše (USLE faktor *R*), koja u prosjeku na godišnjem nivou iznosi 70,87. Dobivena vrijednost faktora *R*, prema kriteriju koji daju Scrinzi i sar. (2006), svrstava Željezno polje u područje niske erozivnosti kiše.

Na osnovu provedenih terenskih istraživanja pri kojima su uzeti prosječni uzorci tla, a potom obavljene laboratorijske analize njihovih fizičkih i hemijskih karakteristika, izračunata je (Formula 3) erodibilnost tla (faktor *K*) za 8 reprezentativnih parcela (Tabela 2). Dobivene vrijednosti faktora *K* se kreću u uskim granicama od 0,04 do 0,06.

Uzme li se u obzir da se pad terena u prosjeku kreće u rasponu od 10 do 50 %, da su proizvodne parcele dugačke od 15 do 60 m, da je vrijednost tolerantne erozije 4 t ha^{-1} god^{-1}, a prosječna erodibilnost tla 0,05, stepen rizika od erozije, za pretpostavljene padove i dužine padina na području Željeznog polja imat će vrijednosti kao što je prikazano u Tabeli 3.

Tabela 3. Stepen rizika od erozije na području Željeznog polja
The soil erosion risk at Željezno polje

Stepen rizika* *Erosion risk*	Dužina padine (m) *Slope length (m)*					
Pad (%) *Slope (%)*	15	20	30	40	50	60
10	**85,8**	**99,0**	121,3	140,0	156,6	171,5
15	162,1	187,1	229,2	264,7	295,9	324,2
20	262,3	302,8	370,9	428,3	478,8	524,5
30	534,2	616,9	755,5	872,4	975,4	1068,5
40	901,7	1041,2	1275,2	1472,4	1646,2	1803,4
50	1364,6	1575,7	1929,8	2228,4	2491,4	2729,2

*Stepen rizika od erozije: < 20 Neznatan; 21 – 50 Mali; 51 – 100 Umjeren; 101 – 200 Visok; 201 – 400 Ekstremni > 400 Katastrofalan (Auerswald i Schmidt, 1986)

Umjeren stepen rizika od erozije javlja se samo na padovima do 10 % i dužini padine do 20 m. Sa druge strane, katastrofalan rizik od erozije javlja se već pri padovima od 20%, ako su padine duže od 40 m. Vrijednosti potencijalna erozije (*RKLS*) već na padinama dužine 30 m i padu od 10 % iznose 4,9 t ha^{-1} god^{-1}, te kao takve prelaze tolerantne vrijednosti. U ovakvim uslovima, gdje se većina poljoprivredne proizvodnje odvija na površinama čiji je pad veći od 15 %, tlo se ne bi smjelo

ostavljati golo, ne zasijano, pogotovo u slučaju kada je orano u pravcu pada terena, a padinu bi trebalo na svakih 10 m prekidati zatravljenim kanalima.
Ako se zasadi malina, najčešće poljoprivredne kulture ovog područja, ne bi obrađivali i podizali u pravcu pada terena (kakva je trenutno praksa), nego po izohipsama - okomito na pad terena (P_i = 0,5), uz zatravnjivanje međurednog prostora (C_{mt} = 0,08), erozija tla bi se na većini parcela ovog područja držala pod kontrolom, odnosno ispod nivoa tolerantne vrijednosti (Tabela 4).

Tabela 4. Procijenjena količina erozionog nanosa (stvarna erozija) pri sadnji po izohipsama uz zatravnjivanje međurednog prostora ($RKLSC_{mt}P_i$)
Soil erosion sediment in the contour lines sowing and grassing row of space

$RKLSC_{mt}P_i$	Dužina padine (m) *Slope length* (m)					
Pad (%) *Slope* (%)	15	20	30	40	50	60
10	0,14	0,16	0,19	0,22	0,25	0,27
15	0,26	0,30	0,37	0,42	0,47	0,52
20	0,42	0,48	0,59	0,69	0,77	0,84
30	0,85	0,99	1,21	1,40	1,56	1,71
40	1,44	1,67	2,04	2,36	2,63	2,89
50	2,18	2,52	3,09	3,57	3,99	4,37

Vrijednosti *LS* faktora, koje su izračunate na bazi izmjerenih nagiba i dužina reprezentativnih parcela, kreću se u rasponu od 3,22 do 13,88 (Tabela 5). Obzirom da su svojstva tla slična na svim parcelama, što rezultira i sličnim vrijednostima faktora *K*, očit je dominantan uticaj topografije – *LS* faktora na produkciju erozionog nanosa na analiziranim parcelama.

Tabela 5. Vrijednosti faktora *K*, *LS* i stvarne erozije (*A*) na 8 reprezentativnih parcela
The values of the factors K, LS and actual erosion (A) for 8 representative parcels

	Lokacija *Location*	*K*	*RK*	*LS*	*A* t ha^{-1} god^{-1}	*A* $_{parcele}$ t god^{-1}	*T* $_{parcele}$ t god^{-1}
1	Ozimine	0,04	3,36	11,05	8,45	1,83	0,87
2		0,05	4,04	10,73	10,27	2,44	0,95
3	Jezerac	0,05	3,65	3,22	3,08	0,35	0,46
4	Strana	0,06	4,35	11,12	12,77	2,12	0,66
5	Padež	0,05	3,94	13,24	12,66	2,75	0,87
6	Jastrebac	0,06	4,43	13,88	15,94	8,53	2,14
7		0,06	4,36	8,53	9,80	0,75	0,31
8	Samari	0,06	4,87	6,94	7,97	3,02	1,52

Na svim reprezentativnim parcelama zasađena je malina (*C* = 0,27). Nisu provedene nikakve mjere konzervacije tla, a obrada se vrši uz i niz nagib terena (*P* = 1). U takvim uslovima, dobivene vrijednosti stvarne erozije (*A*=*RKLSCP*) kreću se od 3,08 do 15,94 t ha^{-1} god^{-1}. Ako uporedimo dobivene vrijednosti sa tolerantnom erozijom od 4 t ha^{-1} god^{-1}, može se primjetiti da jedino parcela 3 (Jezerac), koja se nalazi na najmanjem nagibu (14 %) ima vrijednosti stvarne erozije (3,08 t ha^{-1} god^{-1}) u granicama koje se mogu tolerisati. Na svih ostalih 7 lokacija stvarna erozija je veća od tolerantne, pogotovo u slučaju parcele 6 (Jastrebac). Proračunato na stvarnu površinu svake parcele ($A_{parcele}$), vrijednosti količine erozinog nanosa koje se kreću od 0,75 do 8,53 t god^{-1}, dosta su više u odnosu na tolerantne ($T_{parcele}$) koje se kreću od 0,31 do 2,14 t god^{-1} (Tabela 5). Ovako visoke vrijednosti stvarne erozije (A) ukazuju na potrebu poduzimanja određenih mjera kojima bi se njihove godišnje vrijednosti na ovim lokacijama smanjile do tolerantne vrijednosti (T = 4 t ha^{-1} god^{-1}). Primjena konturne obrade i skraćivanje dužine parcela u slučaju kada se obrada vrši u pravcu pada terena, kao i zatravljivanje međurednih prostora u malinjacima, svakako su neke od mjera koje bi se u tom smislu mogle preporučiti.

U slučaju da se u međurednom prostoru zasada maline tokom cijele godine nalazi travni pokrivač, vrijednost faktora C bi bile niže (C_{mt} = 0,08), a samim time i procijenjene vrijednost (Tabela 6) količine erozionog nanosa ($RKLSC_{mt}$). Samo zatravnjivanjem međurednog prostora, na svim parcelama osim na lokaciji 6 (Jastrebac), količina erozionog nanosa se može držati ispod nivoa tolerantnih vrijednosti. Sličan rezultat se može postići i eventualnim presijecanjem (skraćivanjem) dužine padina (ili redova malina) na dva jednaka dijela ($RKL_{0,5}SC$) (Tabela 6).

Tabela 6. Procijenjena količina erozionog nanosa uz primjenu mjera konzervacije
The soil erosion sediment if conservation measures are applied

	Lokacija *Location*	$RKLSC_{mt}$ t ha^{-1} god^{-1}	$RKL_{0,5}SC$ t ha^{-1} god^{-1}	$RKL_{0,5}SC_{mt}$ t ha^{-1} god^{-1}
1	Ozimine	2,51	5,98	1,77
2		3,04	7,26	2,15
3	Jezerac	0,91	2,18	0,64
4	Strana	3,78	9,03	2,67
5	Padež	3,75	8,95	2,65
6	Jastrebac	4,72	11,27	3,34
7		2,90	6,93	2,05
8	Samari	2,36	5,63	1,67

Ipak, najbolji rezultati postižu se kombinacijom ovih dviju mjera ($RKL_{0,5}SC_{mt}$), kada se vrijednosti stvarne erozije mogu značajno reducirati (0,64 – 3,34 t ha^{-1} god^{-1}) i tako zadržati u prihvatljivim granicama.

ZAKLJUČCI

Na području Željeznog Polja, na kojem je vršena procjena erozije korištenjem Univerzalne jednačine gubitaka tla erozijom - USLE, pretežno su zastupljena smeđa plitka i srednje duboka, vrlo jako do jako kisela, skeletoidna, u povšinskom horizontu dosta humozna tla, praškasto ilovaste do ilovaste teksture. Glavnina poljoprivredne

proizvodnje na ovom području, koje karakteriše vrlo izlomljen reljef, odvija se na parcelama čiji je nagib cca 10 do 50 %, a dužina 15 do 60 m. Najčešće se uzgaja malina koja je zastupljena na većini obradivih površina.
Na osnovu izračunate vrijednosti erozivnosti kiše (USLE faktor R), koja na području Željeznog Polja u prosjeku na godišnjem nivou iznosi 70,87, i prosječne vrijednosti faktora K koja iznosi 0,05, procijenjena vrijednost potencijalne erozije (*RKLS*) već na padinama dužine 30 m i padu od 10 % iznosi 4,9 t ha^{-1} god^{-1} i prelazi tolerantnu vrijednost (4,0 t ha^{-1} god^{-1}). Stepen rizika od erozije je na ovom području veoma visok, pa se katastrofalan rizik od erozije javlja već pri padovima od 20% ako su padine koje se obrađuju duže od 40 m.
Na svim analiziranim parcelama, na kojima se već duži niz godina uzgaja malina, procijenjene vrijednosti stvarne erozije (*RKLSCP)* kreću se od 3,08 do 15,94 t ha^{-1} god^{-1}. Jedino parcela 3 (Jezerac), koja se nalazi na najmanjem nagibu (14 %) ima vrijednosti stvarne erozije (3,08 t ha^{-1} god^{-1}) u granicama koje se mogu tolerisati.
U navedenim okolnostima tlo koje se obrađuje ni pod koju cijenu ne bi smjelo ostavljati golo, ne zasijano, pogotovo kada je preorano u pravcu nagiba padine. Pri tome, posebnu pažnju posvetiti dužini parcela koje se nalaze na većim nagibima. Preporučuje se primjena konturne obrade tla i konturne sjetve/sadnje kad god to veličina i oblik parcele dozvoljavaju. U zasadima maline obavezno zatravnjivanje međurednog prostora.

LITERATURA

Arnoldus, H. M. J. (1977a). Guidelines for watershed managment, Food and Agriculture Organization of the United Nations, Rome.
Arnoldus, H. M. J. (1977b). Methodology used to determine the maximum potential average annual soil loss due to sheet and rill erosion in Morocco. FAO, soil bulletin 34:39-48.
Arnoldus, H. M. J. (1980). An approximation to the rainfall factor in the universal soil loss equation. In: Assessment of Erosion (De Boodt, Gabriels D, ed.), pp 127-132, Wiley, New York).
Auerswald, K., Schmidt, F. (1986). Atlas der Erosionsgefiihrdung in Bayern - Karten zum flachenhaften Bodenabtrag durch Regen. GLA-Fachbericlzte 1: 1-67.
Brink, R. A. , Densmore, J. W., Hill, G. A. (1977). Science 197:625–630..
Dale, T., Carter, V. G. (1955). Topsoil and Civilization (Univ of Oklahoma Press, Norman, OK).
Desmet, P. J. J. and Govers, G. (1996). A GIS procedure for automatically calculating the USLE LS factor on topographically complex landcape units. Journal of soil and water conservation 51, p. 427-433.
Eswaran, H., R. Lal, P. F. Reich (2001). Land degradation: an overview. Responses to Land Degradation. Proc. 2nd. International Conference on Land Degradation and Desertification. New Delhi, India: Oxford Press. Retrieved 2012-02-05.

FAO (2013). Food Balance Sheets. FAOSTAT, Food and Agriculture Organization of the United Nations, 2004.

Fournier, F. (1960). Climat et erosion, la relation entre erosion dul sol par “1” eau et les precipitations atmospheriques, presses Universitaries de Frame, Paris.

Hooke, R. L. (2000). Geology, 28:843–846

Kelvin K. K., Darrien Y. S., Chiu P. C. (2013). Journal of Water Resource and Protection, 5, 1149-1154. http://dx.doi.org/10.4236/jwarp.2013.512121.

Lowdermilk, W. C. (1953). Conquest of the Land Through 7,000 Years (US Department of Agriculture, Washington, DC.

Merritt, W. S., Croke, B. F. W., Jakeman, A. J., Perez, P., Letcher, R. A., Buller, C. (2001). The Biophysical Toolbox: a Biophysical Modelling Tool Developed Within the IWRAM-DSS. iCAM Working Paper, Thailand.

Morgan, R. P. C. (1996). Soil erosion and conservation. Addison-Wesley Boston MA.

Pimentel, D., Harvey, C., Resosudarmo, P., Sinclair, K., Kurz, D., McNair, M., Crist, S., Sphpritz, L., Fitton, L., Saffouri, R. (1995). Environmental and Economic Costs of Soil Erosion and Conservation Benefits. 267, 1117–1123.

Pimentel, D. (2006). Soil Erosion: A Food and Environmental Threat Environment. Development and Sustainability Volume 8, Issue 1, 119–137.

Pimentel, D. Burgess, M. (2013). Soil Erosion Threatens Food Production, Agriculture, 3, 443-463.

Pimentel, D., Allen, J., Beers, A., Guinand, L., Linder, R., McLaughlin, P., Meer, B., Musonda, D., Perdue, D., Poisson, S. (1987). BioScience 37: 277–283.

Renard, KG, Foster, GA, Weesies, GA, McCool, DK (1997). Predicting Soil Erosion by Water: A Guide to Conservation Planning with the Revised Universal Soil Loss Equation (RUSLE).'Agriculture Handbook No: 703: USDA: Washington, DC.).

Resulović, H., Čustović, H., Čengić, I. (2008). Sistematika tla/zemljišta. Univerzitet u Sarajevu.

Robert, P., Stone, P. (2012). Factsheet, Universal Soil Loss Equation (USLE), Order No. 00-001, 12-051.

Sample, I. (2007). Global Foods Crisis Looms as Climate Change and Population Growth Strip Fertile Land". The Guardian.

Schaub, D. and Prasuhn V. (1998). A Map of Soil Erosion on Arable Land as a Planning Tool for Sustainable Land Use in Switzerland. Advances in GeoEcology 31.

Scrinzi, G., Gregori, E., Giannetti, F., Galvan, D., Zorn, G., Colle, G., Andreanelli, M. C. (2006). Un modello di valutazione della funzionalità protettiva del bosco per la pianificayione forestale: la componente stabilità dei versanti rispetto ai fenomeni franosi superficiali. 3, 1, 98-155.

Selby, M. J. (1993). Hillslope Materials and Processes, Oxford Univ Press, Oxford.

Shaler, N. S. (1905). Man and the Earth (Fox, Duffield, NY).

SROŽ (2011). Strategija razvoja općine Žepče za period 2011. – 2018. Općina Žepče.

Ursic S. J., Dendy, F. E. (1965). Proceedings of the Federal Inter-Agency Sedimentation Conference, 1963 (US Department of Agriculture, Washington, DC), 47–52.

USDA (2008). Field Office Technical Guide - Section I – Erosion Prediction. New York.

Verheijen F. G. A., Jones R. J. A., Rickson R. J., Smith C. J. (2009). Tolerable versus actual soil erosion rates in Europe. Earth-Science Reviews, 94 (1-4) , 23-38.

Wischmeier, W. H., Smith, D. D. (1978). Predicting rainfall erosion losses. A guide to conservation planning. United States Department of Agriculture, Washington, D.C.

Wolman, M. G. (1967). Geogr. Ann. 49A: 385–395.

Young, A. (1998). Land Resources: Now and for the Future; Cambridge University Press: Cambridge, UK.

Žurovec J., Čadro, S. (2008). Erosion Risk on the Arable Soils on the Hill Area of Canton Sarajevo, Radovi Poljoprivrednog Fakulteta Univerziteta U Sarajevu, Br.59/2, Sarajevo.

EFFECTS OF OREGANO AND GARLIC ESSENTIAL OILS ON HOLSTEIN CALVES PERFORMANCE AND SOME BLOOD PARAMETERS*

Hayrullah Bora Ünlü[1]

Original scientific paper

Summary

The calves were evaluated for the effects of oregano (*Oregano onites L.*) and garlic essential oils (*Allium sativa Lillaceae*) supplementation in whole milk (250 mg/calf/day) during the first 6 weeks on growth performance, feed consumption, feed conversion ratio, fecal score, incidence of scours, the count of *Escherichia coli* and *Lactobacillus spp.* in gaita, body measurements as well as on some blood parameters. At the end of the experiment, there were no effects of oregano and garlic essential oils supplementation in whole milk on daily gain, whole milk, calf starter, total feed consumption, feed conversion ratio, body measurements, gaita bacteria counts and blood parameters (P>0.05). Meanwhile, essential oils showed no significant effects on counts of fecal coliform, *Escherichia coli* and *Lactobacillus spp*., fecal fluidity score, days with scours, and number of treatment days for scours (P>0.05). Also there were no significant differences among groups in total protein, albumin, globulin, urea and bilirubin in blood serum, hemoglobin, erythrocyte, leukocyte, basophile, lymphocyte and monocyte in blood (P>0.05). However, oregano and garlic essential oils supplementation reduced significantly gaita total coliform count (P<0.05). Adding garlic essential oil to whole milk decreased blood serum total cholesterol level (P<0.05). Although oregano essential oil supplementation had biologically better calf performance, feed consumption and feed conversion efficiency, these results were not significant statistically.

Key words: *calves, oregano essential oil, garlic essential oil, performance, blood parameters*

INTRODUCTION

Calves born on a dairy farm represents an opportunity to maintain or increase herd size, to improve the herd genetically or to improve economic returns to the farm. The objectives of raising the newborn calf to weaning age are optimizing growth and minimizing health problems (Heinrichs and Jones, 2003). Because of this, caring on feeding and health management practices are important factors in raising healthy

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Ege University, Faculty of Agriculture, Department of Animal Science, İZMİR-TURKEY
Correspondence Author: Hayrullah Bora Ünlü, hayrullah.bora.unlu@ege.edu.tr

calves. Unfortunately, the inadequate development of the immune system of newborn calves increases the risk of disease and the highest mortality rate is seen in this period (Roy, 1980). Since the discovery and development of the first antibiotics, these drugs have played an important role in treatment of disease in humans and animals. Experiments conducted in 1946 showed that low, sub therapeutic levels of antibiotics could increase feed efficiency and growth in animal production. After that, there has been widespread incorporation of antibiotics into animal feeds in many countries (Khachatourians, 1998). Antibiotics have been used widely as animal feed additives for the past several decades to improve health and performance of animals (Uzmay *et al*., 2011). However, the extensive use of antibiotics can result in resistance to antibiotic pathogens, occurring a potential risk of exposure for animals and humans, in whom they cause disease that, can not be treated by conventional antibiotics (Friedman, 2007).

There is an increasing public and scientific concern about the use of antibiotics as feed additives in animal production. This concern is fuelled by the emergence of antibiotic resistance in many human pathogenic bacteria (Parveen *et al*., 2006), the release of contaminating residues into the environment (Yang and Carlson, 2004), and the risk that growth promoting antibiotic residues may occur in foods which are animal origin. For all these reasons, the European Union (EU) decided that antibiotics used in livestock as production enhancers would be banned from 1 January 2006 (EU regulation no.1831/2003 of the European Parliament and of the Council of 22 September 2003). This ban effectively ends nearly fifty years of antibiotic use for non-therapeutic purposes. It covers all classes of antibiotics, including ionophores, a group of substances extensively used as coccidiostats in poultry production and as growth promoters or production enhancers in ruminants. Consequently, there is nowadays a real demand among animal producers for alternative feed additives and among consumers for more natural and safe products in the human food supply chain. Therefore there is a great renewed interest in developing natural alternative supplements to maintain animal performance and wellbeing.

In an effort to eliminate antibiotics from animal feeds, many additives have been proposed for addition to calves feeds. Plants produce an extensive variety of organic compounds derived from their seconder metabolites that are classified in three main groups; saponins, tannins and essential oils. Some essential oils have antimicrobial activities and are currently considered to be safety for human and animal consumption, and are categorized as GRAS (Generally Recognized as Safe; FDA, 2004) in the USA, Their potential use in ruminants has been recently reviewed by Calsamiglia *et al*., (2007) and Benchaar *et al*., (2007).

Some essential oils/compounds demonstrated good potential; including efficacy, tolerance to low pH, and selectivity towards bacterial pathogens in reducing human and animal bacterial pathogens in intestinal tract (Si *et al.*, 2006). But there are few studies about effects of oregano essential oils and garlic oils on calves performance and health. Carvacrol and thymol are monoterpenoids with a strong antimicrobial activity against a wide range of gram-positive and negative bacteria, those are found in oregano (*Origanum* spp.). Numerous *in vitro* studies demonstrated that essential oil including thymol, carvacrol, *etc*., displayed antimicrobial activity against intestinal microbes such as *C. perfringens, S. typhimurium* and *E. coli* (Helander *et al*., 1998; Hammer *et al*., 1999). Antimicrobial action of essential oil is mediated by lipophilic property to perforate the bacterial membrane, which releases membrane components from the cells to the external environment (Helander *et al*., 1998). The small molecular weight of thymol and carvacrol allows them to gain access to the cell membrane through the pores of the external wall, as a result of the strong and wide-spectrum activity of essential oils against bacteria reported by Castillejos *et al*., (2006). Garlic oil is a mix of a large number of different molecules that are found in the plant or are the result of changes occurring during oil extraction and processing (Lawson, 1996). Although garlic oil is known for its therapeutic properties, its antimicrobial activity Harris (2001) against a wide spectrum of gram-positive and negative bacteria is its most prominent activity and has been thoroughly studied (Reuter *et al*., 1996). There are only a few reports on the effects of garlic extract on animal performance.

The objective of this study was to investigate the effect of oregano and garlic essential oils mixed in the whole milk on growth and health of Holstein calves.

MATERIALS AND METHODS

Thirty six Holstein calves (18 male, 18 female) born in the Agricultural Faculty Farm of Ege University, were randomly assigned within sex, to one of three groups at 2 d of age.

In the study, there are three experimental groups and each group has 12 calves, including randomly distributed 6 females and 6 males calves. Experiment was carried out in individual calf pens. After the birth, calves separated from their dams and fed colostrum for 3 d. On the 2nd day, calves were switched to their respective liquid diets.

Two essential oils, derived from selected *Origanum onites* L. and *Allium sativum* L., growing wild in Turkey were used. Oregano essential oil obtained by steam distillation from selected *O. onites* ssp. was provided by Türer Agriculture Ltd Company (İzmir, Turkey). The carvacrol and thymol contents, which are the most active compounds of oregano essential oil, were determined at 77.33% and 9.64% respectively. Garlic essential oil obtained by steam distillation from selected *A. sativum* L. growing wild in Turkey was used and provided by Konsa Concentrate Industry Inc. (İstanbul, Turkey). The 2-propenyl thioacetonitryl, trisulphide methyl 2-propenyl and disulphide di-2-propenyl contents, which are the most active compounds of garlic essential oil, were determined at 43.25%, 23.40% and 20.87% respectively. Calves were fed whole milk (control) and whole milk supplemented with 250 mg/d oregano essential oil and whole milk supplemented with 250 mg/d garlic oil per calf daily at a.m. feeding. The essential oils used in the experiment were diluted 1/10 in 96% food ethanol to provide more homogeneous mixing and ease of use with whole milk. The experiment continued for 6 weeks. Calves were fed 4 L of whole milk per day from a bucket in two equal feedings at 08:00 and 18:00 h for 6 weeks. Whole milk consumption was monitored daily. Pelleted calf starter and fresh water were available *ad libitum* during the experiment (from d 2 to 43 of age). Intake of calf starter was measured regularly. No hay was offered throughout the experiment. Calves were weighed at the beginning of the experiment and then weekly till the end of the experiment. Height at withers, heart girth, withers to pins, and point of shoulder to pins were measured at the beginning and at the end of the experiment. Fecal fluidity scores were monitored daily according to Larson *et al*., (1977).

Samples of milk and starter were collected at different periods of the experiment for analysis of nutrient composition. Samples of starter were analyzed for dry matter (DM, 89.88%), crude protein (15.19%), ether extract (3.69%), crude fiber (9.92%, the Lepper method), and crude ash (7.25%) using the Weende method (AOAC, 1995). Metabolic energy of starter feed as Kcal/kg in DM (2499 kcal/kg) was determined using a prediction equation TSI-9610 (1991). Whole milk samples were analyzed for dry matter (12.57%, DM) and ash (0.72%) using the Gravimetric method, for protein (3.37%) using the Kjeldahl method, for fat (3.69%) using the Gerber method, and for lactose (4.79%) using the polarimetric method (Oysun, 2001). The active ingredients of the oregano and garlic essential oils were determined by GC / MS (HP 6890 GC / 5973 MSD) by U.S.A. Pharmacopeia National Formulary methods USP 23 NF 18 (1995). After the birth, on the morning of the 7th day of the experiment, a fresh fecal sample was taken from each calves (20-30 g) from the rectum to the gaita containers for microbiological analysis. *Lactobacillus spp.* counts in calf faces were made using MRS agar by the bulk plate method. Total *Coliform*, *Fecal Coliform* and *Escherichia coli* counts in fecal samples were carried out according to TSI-6063-ISO-7251 (1996). On the morning of the 43rd day of the experiment, the blood sample was taken for the biochemical and hematological counting of the *vena jugularis*. Blood biochemical values were determined by filtered photometric method on the Otecnica BT-2000 Plus. Abbott Cell Dyn 3500 was used for determining hematologic values of the blood samples by impedance and optical method. Hemoglobin values of the blood samples were measured by the spectrophotometric.

Data were subjected to ANOVA using General Linear Models (SPSS, 2006). The following ANOVA model was used to analyze intake, growth, feed efficiency, blood parameters, bacteria counts and calf scours data. Initial body weight was used as a covariate in the analysis body weight and body weight gains for various periods. The number of microbial colony forming units (CFUs) was expressed as logarithmic (log10) transformation per gram of intestinal gaita. All microbiological concentrations from each calves were subjected to log transformation prior to statistical analysis.

RESULTS AND DISCUSSION

Means and standard errors for whole milk, calf starter and total DM intakes of calves for various age periods are given in Table 1. Whole milk was fed to calves in all groups were at a constant amount (4 L/calf/d) for 6 weeks. Few acceptability problems were observed.

Table 1. Means and standard errors for whole milk, calf starter and total DM intakes of calves for various age periods

Group	Control	Oregano	Garlic	SE	*P*
DM intake from whole milk, g/calf/day					
0-3 w	478.98	490.24	461.72	9.91	0.150
3-6 w	502.80	502.80	502.80	0.00	-
0-6 w	490.89	496.52	482.25	4.95	0.150
DM intake from starter, g/calf/d					
0-3 w	56.30	69.53	66.55	12.25	0.725
3-6 w	359.64	423.96	397.59	53.55	0.691
0-6 w	207.97	246.75	232.07	31.36	0.675
Total DM intake, g/calf/d					
0-3 w	535.28	559.77	528.26	17.28	0.412
3-6 w	862.44	926.76	900.39	53.55	0.691
0-6 w	698.86	743.27	714.33	32.10	0.608

Dry mater intake of whole milk was similar in all groups. Dry matter intakes from milk for w 0 through 6 were 490.89, 496.52 and 482.25 g/d for calves in control, oregano and garlic essential oils groups respectively. However, milk intake was not influenced by the dietary oregano and garlic essential oils supplementation ($P>0.05$) (Table 1). Calf starter dry matter intakes were similar in all groups for various age periods ($P>0.05$). Calves in oregano treatment consumed 17.8% more calf starter than calves in control and 6.6% more than calves in garlic oil supplemented group (423.96, 359.64 and 397.59 g/d) for w 3 through 6. The differences are not statistically significant but proportional levels seem biologically meaningful. This suggests that oregano affects the desire for consumption positively. The findings about whole milk dry matter consumption and starter intake of treatment groups are consistent with the findings of the relevant studies Greathead *et al*., (2000) and Soltan (2009). On the other hand, in a recent study by Ghosh *et al*., (2010) demonstrated that garlic extract supplementation to whole milk significantly increased dry matter intake of calves.

Mean initial BW (2 d of age) in control, oregano and garlic essential oils supplemented groups were 41.01, 41.25 and 43.79 kg, respectively (Table 2). Mean BW at w 3 (45.37, 47.44 and 46.54 kg) and at w 6 (56.85, 59.52 and 58.34 kg) did not differ among the treatment groups ($P>0.05$). Mean daily BW gains from w 0 to 3, w 3 to 6, and w 0 to 6 were also unaffected by treatment ($P>0.05$). However, calves in oregano treatment gained 17.9% more BW than control; 7.2% more BW than garlic group from w 0 to 6. Tendency for improved BW gain in oregano supplementation during 0 to 6 w of age might have resulted from increased starter intake. Especially, the oregano group showed biologically a higher live weight gain with a comparison to control and garlic groups in all periods from the first week of the treatment. This may be due to the addition of oregano essential oil's role on improving the calves health. On the other hand, calves in garlic group were not observed a stable body weight gain as oregano supplementation at all age periods. However, Greatheat *et al.,* (2000) and Ghosh *et al*., (2010) reported that addition of garlic extract increased body weight gain significantly in calves. Also, Ahmed *et al*., (2009) found that addition of 2.5% natural garlic juice significantly increased daily gain of the growing buffalo calves compared with the control group but did not significantly affect the feed consumption compared with the control.

Feed efficiencies (DM intake/BW gain) were not different among the all groups for various age periods (Table 2) ($P>0.05$). These values were observed to be very close to each other in all periods for treatment groups. Similarly, Soltan (2009) also found that addition of essential oils to starter diet did not change feed efficiency in calves. However, Greathead *et al*., (2000); Marsh (2006) and Ghosh *et al*., (2010) reported that addition of essential oils or extract to milk or milk replacer improved feed efficiency in calves. This may be due to the quality of the calf starter. In the 3-6 week period when the starter feed consumption increased, the low feed quality did not reflect positively on the live weight gain. For this reason, there may not be any improvement in the feed efficiency. This situation can be understood clearly because the oregano treatment which had the highest feed consumption but had the same feed efficiency value as the other two groups in 3-6 w age period.

Skeletal growth (height at withers, heart girth, withers to pins, and point of shoulder to pins) was similar for calves in all groups ($P>0.05$) (Table 2). The fact that the body measurements were very close to each other among the groups, suggests that skeletal development in the calves has developed at similar levels.

The effect of oregano and garlic essential oils supplementation on coliform counts, fecal coliform counts, *E. coli* and *Lactobacillus spp*. in the faces of calves at 7 day age old are shown in Table 3. There were no supplementation effects on fecal coliform counts, *E. coli* and *Lactobacillus spp*. counts in the treatment groups. The highest Coliform bacteria counts were obtained from control ($P<0.05$). Coliform counts were significantly decreased in faces of calves in oregano and garlic essential oils groups compared with control. Similarly Ghosh *et al.,* (2010) found that fecal coliform count was reduced when calves were fed additive garlic extract. Also, numerous in vitro studies demonstrated that essential oil including thymol, carvacrol and allicin displayed antimicrobial activity against intestinal microbes such as *C. perfringens*, *S. typhimurium* and *E. coli* (Rees *et al.,* 1993; Helander *et al.,* 1998; Hammer *et al.,* 1999). Antimicrobial action of essential oil is mediated by lipophilic property to perforate the bacterial membrane, which releases membrane components from the cells to the external environment (Helander *et al.,* 1998). Also the findings in this study, oregano and garlic essential oils showed selective antimicrobial effect against to beneficial bacteria in the digestive system of the calves such as *Lactobacillus spp.*, has not been adversely affected. The results not only confirmed the activity of these essential oils against coliform, but also demonstrated their selectivity towards the pathogen with little effect on beneficial gut bacteria (Si *et al.,* 2006). Rees *et al.,*

(1993) and Si *et al.*, (2006) declared that lactic acid bacteria are the one of the most resistant microorganisms to the inhibitory effects of oregano and garlic essential oils. Studies carried out with calves seem to confirm the in vitro findings.

Table 2. Means and standard errors for body weight, body weight gains, feed efficiencies, body measurements of calves for various age periods

Group	Control	Oregano	Garlic	SE	*P*
BW, kg					
d 2 (initial BW)	41.01	41.25	43.79	1.40	0.318
w 3	45.37	47.44	46.54	0.76	0.170
w 6	56.85	59.52	58.34	1.31	0.360
BW gain, g/calf/d					
0-3 w	160.07	258.27	215.37	36.33	0.101
3-6 w	546.62	575.21	562.29	45.04	0.902
0-6 w	353.35	416.74	388.83	31.27	0.362
Feed efficiency, kg DM/kg BW gain					
0-3 w	2.70	2.41	2.49	0.34	0.839
3-6 w	1.57	1.68	1.64	0.07	0.620
0-6 w	2.09	1.92	1.98	0.11	0.544
Height at withers, cm					
d 2	77.36	78.32	78.44	0.84	0.614
d 43	85.53	84.46	86.77	1.00	0.286
Heart girth, cm					
d 2	79.452	80.136	81.64	0.64	0.074
d 43	89.147	91.955	91.55	1.01	0.120
Withers to pins, cm					
d 2	54.29	53.75	55.20	0.76	0.424
d 43	60.53	61.77	62.09	0.91	0.455
Point of shoulder to pins, cm					
d 2	70.19	70.08	71.35	1.01	0.635
d 43	80.67	80.79	81.79	0.98	0.694

Table 3. Means and standard errors for fecal microbiology counts of seven day age calves

Bacteria Types	Control	Oregano	Garlic	SE	*P*
Coliform Log EMS/ml	13.88^{b}	12.23^{a}	12.24^{ab}	0.49	0.05
Fecal Coliform Log EMS/ml	12.12	11.02	11.86	0.56	0.40
E. coli Log EMS/ml	11.30	10.59	10.98	0.27	0.25
Lactobacillus spp. Log cfu/ml	9.99	9.88	9.89	0.36	0.97

*a,b Means within a column in each variable with no common superscript differ significantly (P≤0.05).

In the present study, addition of essential oil to whole milk did not affect serum biochemical and hematology blood values of calves (Table 4). The lowest serum cholesterol value was obtained from garlic oil treatment group (P<0.05). The serum cholesterol value was significantly decreased in calves from garlic oil compared with calves from control and oregano group. However, the calf serum cholesterol levels in blood of calves is associated with the milk dry matter and milk fat content consumed calves (Moody *et al.*, 1992), all calves in the treatment groups consumed same whole milk. Also calves serum cholesterol levels in all groups are in the reference range (1.2-3.8 mmol/L) for calves as reported by Lumsden *et al*., (1980); Mohri *et al*., (2007). Similarly, Gebhardt (1993) and Orekhov *et al*., (1995) reported that garlic oil or extracts reduce cholesterol and cholesterol esters in blood serum by inhibiting enzymatic steps in cholesterol biosynthesis. The findings of this study showed that garlic oil has hypocholesterolemic effect on blood serum cholesterol level for calves.

Table 4. Means and standard errors for biochemical and hematological blood values of calves

	Control	Oregano	Garlic	SH	*P*
Total Protein, g/L	57.33	54.50	54.33	1.73	0.398
Albumin, g/L	33.08	31.75	32.83	0.71	0.404
Globulin, g/L	24.25	22.75	21.50	1.87	0.587
Urea, g/L	2.07	1.89	2.11	0.15	0.560
Cholesterol, mmol/L	1.47^{ab}	1.66^{b}	1.24^{a}	7.76	0.003
Bilirubin, mmol/L	3.67	3.17	3.17	0.28	0.232
Hemoglobin, g/L	85.81	89.39	86.37	3.90	0.785
Erythrocyte, M/mm^3	7.92	8.44	8.64	0.31	0.264
Leukocyte, M/mm^3	6.65	6.48	6.43	0.54	0.957
Basophil, M/mm^3	0.12	0.08	0.13	0.02	0.408
Lymphocyte, M/mm^3	2.77	2.85	2.53	0.42	0.858
Monocyte, M/mm^3	1.07	1.20	1.15	0.14	0.802

*a,b Means within a column in each variable with no common superscript differ significantly (P<0.05).

There were no differences in fecal fluidity scores for w 0 to 3, w 3 to 6, and w 0 to 6 between the groups in the study (Table 5). Calf days with scours and calf days treated for scours in various age periods were also unaffected by treatment. Calf days with scours and calf days treated for scours in oregano essential oil group for w 0 to 6 was numerically lower than control and garlic group. Nevertheless, calf scours were a common problem especially during the first 3 weeks of the experiment as seen in Table 5. The values of the calf days with scours and days treated for scours obtained in this study were compatible results reported with Bampidis *et al*., (2006) and Greathead *et al*., (2000). But Ishihara *et al*., (2001) and Ghosh *et al*., (2010) demonstrated that fecal scores and days with scours were reduced when calves were fed with additive containing herbal green tea and garlic extract. It is clear that the addition of oregano essential oil to whole milk tended to decrease the rate of calf scour, number of the days with diarrhea and days of treatment. Because of the biologically increasing live weight, feed consumption and an improvement in feed efficiency during the 0-3 week period, oregano essential oil supplementation is considered to have a positive effect on the health of calves. Lower fecal score, lower days with scours, lower days treated for scour and lower coliform count in the oregano group are thought to be originated from the antimicrobial properties of oregano essential oils.

Table 5. Means and standard errors for fecal score, days with scours and days treated of calves for various age periods

Group	Control	Oregano	Garlic	SE	*P*
Fecal fluidity score					
1 w	1.94	1.76	1.92	0.16	0.685
2 w	2.78	2.31	2.65	0.21	0.287
3 w	1.33	1.14	1.22	0.12	0.580
0-3 w	2.02	1.73	1.93	0.12	0.246
3-6 w	0.99	1.01	1.01	0.01	0.173
0-6 w	1.50	1.37	1.47	0.06	0.286
Calf days with scours,%[2]	5.95	4.65	5.38	0.74	0.462
Calf days treated for scours,%[3]	5.57	3.07	4.77	0.77	0.077

[1]Fecal fluidity score, 1=normal, 2=soft, 3=runny, 4=watery;[2] Days with a fecal fluidity score of ≥3; [3] Days treated for scours.

CONCLUSIONS

In conclusion, addition of oregano and garlic essential oils to warm whole milk did not affect feed intake, BW gain, skeletal growth, feed efficiency, fecal coliform counts, *E. coli* and *Lactobacillus spp*. counts in the faces, serum biochemical values, blood hematology values, fecal fluidity score, calf days with scours, and calf days treated for scours in Holstein calves in a 6-week experiment. However, calf health, starter intake and consequently BW gain from 0 to 3 w of age tended to be improved in calves in oregano group compared with calves in control and garlic group. Oregano and garlic essential oils have antimicrobial properties against the coli bacteria on the calves. Also, there are no adverse effects on useful bacteria such as lactobacillus. Oregano essential oil supplementation which has biologically positive effect on the performance and health of calves can be evaluated as a natural feed additive potential.
More detailed studies on oregano and garlic essential oils, especially including dose experiments need to be carried out in order to present their significant effects on performance and health of calves.

REFERENCES

Ahmed, A. A., Bassuony, N. I., Awad, E. S., Aiad, A. M., Mohamed, S. A. (2009): Adding natural juice of vegetables and fruitage to ruminant diets (B) nutrients utilization, microbial safety and immunity, effect of diets supplemented with lemon, onion and garlic juice fed to growing buffalo calves. World Journal of Agricultural Sciences, 5(4):456-465.

AOAC, (Association of Official Analytical Chemists). (1995): Official Methods of Analysis, 16th Edition, Edited by Patricia Cunniff. Virginia, USA.

Bampidis, V. A., Christodoulou, V., Florou-Paneri, P., Christaki, E. (2006): Effect of dried oregano leaves versus neomycin in treating newborn calves with colibacillosis. J. Vet. Med. A., 53:154–156.

Benchaar, C., Calsamigli, S., Chaves, A. V., Fraser, G. R., Colombatto, D., McAllister, T. A., Beauchemin, K. A. (2007): A review of plant-derived essential oils in ruminant nutrition and production. Anim. Feed Sci. Technol., 145:209-228.

Calsamiglia, S. M., Busquet, P. W., Cardozo, L., Castillejos, L., Ferret, A. (2007): Essential oils for modifying rumen fermentation: A review. J. Dairy Sci., 90:2580-2595.

Castillejos, L., S. Calsamiglia, Ferret A. (2006): Effect of essential oils active compounds on rumen microbial fermentation and nutrient flow in in vitro systems. J. Dairy. Sci., 89:2649-2658.

FDA, (2005): Freedom of information summary. Supplemental new animal drug application. NADA 095-735. Monensin Sodium (RUMENSIN 80): Type A Medicated Article for Dairy Cattle. http://www.fda.gov/cvm/FOI/095-735s121505.pdf

Friedman, M., Henika, P. R., Levin, C. E., Mandrell, R. E. (2004): Antibacterial activities of plant essential oils and their components against *Escherichia coli* O157:H7 and *Salmonella enterica* in apple juice. J. Agric. Food Chem., 52:6042–6048.

Gebhardt, R. (1993): Multiple inhibitory effects of garlic extracts on cholesterol biosynthesis in hepatocytes. Lipis 28(7):613-619.

Ghosh, S., Mehla, R. K., Sirohi, S. K., Roy, B. (2010): The effect of dietary garlic supplementation on body weight gain, feed intake, feed conversion efficiency, faecal score, faecal coliform count and feeding cost in crossbred dairy calves. Trop. Anim. Health Prod., 42:961–968.

Greathead, H. M. R., Forbes, J. M., Beaumont, D., Kamel, C. (2000): The effect of a formulation of natural essential oils used as an additive with a milk replacer and a compound feed on the feed efficiency of calves. British Society of Animal Sciences Annual Winter Meeting.

Harris, J. C., Cottrell, S. L., Plummer, S., Lloyd, D. (2001): Antimicrobial properties of *Allium sativum* (garlic). Applied Microbiol Biotechnology, 57:282–286.

Heinrichs, A. J., Jones, C. (2003): Feeding the newborn dair calf. The Pennsylvania

State University, 328 Boucke Building, University Park, PA 16802-5901, Tel 814865-4700/V, 814-863-1150/TTY, CAT UD013 5M8/03:3434.

Hammer, K. A., Carson, C. F., Riley, T. V. (1999): Antimicrobial activity of essential oils and other plants extracts. Journal of Applied Microbiology, 86:985–990.

Helander, I. M., Alakomi, H. L., Latva, K. K., Mattila, S. T., Pol, I., Smid, E. J., Gorris, L. G. M., Von Wright, A. (1998): Characterization of the action of selected essential oil components on gram-negative bacteria. Journal of Agricultural and Food Chemistry, 46:3590–3595.

Ishihara, N., Chu, D. C., Akachi, S., Juneja, L. R. (2001): Improvement of intestinal microflora balance and prevention of digestive and respiratory organ diseases in calves by green tea extracts. Livestock Production Science, 68:217–229.

Khachatourians, G. G. (1998): Agricultural use of antibiotics and the evolution and transfer of antibiotic resistant bacteria. Can. Med. Assoc. J., 159:1129–1136.

Larson, L. L., Owen, F. G., Albight, J. L., Appleman, R. D., Lamb, R. C., Muller, L. D. (1977): Guidelines toward more uniformity in measuring and reporting calf experimental data[1]. Journal of Dairy Science, 60: 989-991.

Lawson, L. (1996): The composition and chemistry of garlic cloves and processed garlic. in Garlic. The Science and Therapeutic Application of *Allium sativum L*. and Related Spieces. Koch H.P., Lawson L.D., Williams & Wilkins, Baltimore, MD: 37-107.

Lumsden, J. H., Mullen, K., Rowe, R. (1980): Hematology and biochemistry reference values for female Holstein cattle. Can. J. Comp. Med., 44:24-31.

Marsh, S. (2006): Calves fed an essential oil as part of their early weaning concentrate ration produced significantly higher live weight gains and recorded lower scour scores than a control group in a recent Harper Adams University College monitored trial, http://flavex.co.uk/animalfeed/news/42

Moody, D. E., Hohenboken, W. D., Beal, W. E., Thye, F. W. (1992): Concentration of plasma cholesterol in beef cows and calves, milk production and calf gain. Journal of Anim. Science, 70:1464-1470.

Mohri, M., Sharifi, K., Eidi, S. (2007): Hematology and serum biochemistry of Holstein dairy calves: age related changes and comparison with blood composition in adults. Research in Veterinary Science, 83:30–39.

Orekhov, A. N., Tertov, V. V., Sobenin, A., Pivovarova, E. M. (1995): Direct anti-atherosclerosis related effects of garlic. Annals of Medicine, 27:63-65.

Oysun, G. (2001): Methods of analysis for milk and milk products. 3rd ed. Ege Uni. Ziraat Fak. Yay. No: 504. E.Ü.Z.F. Ofset Atelyesi, Izmir-Turkey [in Turkish].

Parveen, S., Lukasik, J., Scott, T. M., Tamplin, M. L., Portier, K. M., Sheperd, S., Braun, K., Farrah, S. R. (2006): Geographical variation in antibiotic resistance profiles of E. coli isolated from swine poultry beef and dairy cattle farm water retention ponds in Florida. Journal of Applied Microbiology, 100:50–57.

Reuter, H. D., Koch, J. P., Lawson, L. (1996): Therapeutic effects and applications of garlic and its preparations in Garlic. The Science and Therapeutic Application of *Allium sativum L*. and Related Species. Koch H.P., Lawson L.D., Williams

& Wilkins, Baltimore, MD: 135-212.

Rees, L. P., Minney, S. F., Plummer, N. T., Slater, J. H., Skyrme, D. A. (1993): A quantitative assessment of the Trop Anim Health Prod (2010) 42:961–968 967 antimicrobial activity of garlic (*Allium sativum*). World Journal of Microbiology and Biotechnology, 9:303–307.

Roy, J. H. B. (1980): Factors affecting susceptibility of calves to disease. Journal of Dairy Science 63(4): 650-663.

Si, W., Gong, J., Tsao, R., Zhou, T., Yu, H., Poppe, C., Johnson, R., Du, Z. (2006): Antimicrobial activity of essential oils and structurally related synthetic food additives towards selected pathogenic and beneficial gut bacteria. Journal of Applied Microbiology, 100:296–305.

SPSS, (2006): SPSS for Windows evaluation version. 15.0, Chicago, IL, USA.

Soltan, M. A. (2009): Effect of essential oils supplementation on growth performance, nutrient digestibility, health condition of Holstein male calves during pre-and post-weaning periods. Pakistan Journal of Nutrition, 8(5):642-652.

TSI,. (1991): Turkish Standard Institute, Animal feeds-metabolizable energy determination (chemical method). TSI number: 9610, Ankara, Turkey. [in Turkish].

TSI,. (1996): Microbiology-the general guilance for enumeration of presumptive Escherichia coli-most probable number technique. TSI, Necatibey Caddesi, Bakanlıklar-ANKARA. [in Turkish].

U.S.A, Pharmacopeia National Formularty. (1995): USP 23 NF 18:1755.

Uzmay, C., Kılıç, A., Kaya, I., Özkul, H., Önenç, S. S., Polat, M. (2011): Effect of mannan oligosaccharide addition to whole milk on growth and health of Holstein calves. Archiv Tierzucht, 54(2):127-136.

Yang, S., Carlson, K. (2004): Routine monitoring of antibiotics in water and wastewater with a radio immuneassay technique. Water Research, 38:3155–3166.

NUTRITIVE EVALUATION AND PREDICTION OF CHOCOLATE AS UNUSUAL FEEDSTUFF FOR RUMINANT BY NEAR-INFRARED REFLECTANCE SPECTROSCOPY (NIRS)*

Hayrullah Bora Ünlü[1], Mürsel Özdoğan[2], Figen Kırkpınar[1], Hülya Özelçam[1]

Original scientific paper

Summary

Chemical analysis (CAM) and near-infrared reflectance spectroscopy (NIRS) were used to predict the nutritive characteristics of 36 chocolate samples as an unusual feedstuffs which were manufactured for human consumption but which are no longer intended for human consumption due to problems of manufacturing or expiration of internal sell-by date. Firstly chocolate samples were analyzed the chemical composition, mineral contents, *in vitro* dry and organic matter digestibility (DMD-OMS), metabolizable energy (ME) and then these analysis values were calibrated and predicted by NIRS.

DM, CA, OM, CP, EE, CF, sugar, starch, NSC, NDF, ADF, ADL, DMD, OMD, ME_{nc}, ME_e and mineral contents of the chocolates samples which were analyzed by both techniques. Also the coefficient of multiple determination (R^2); the value of the root mean square error of cross validation (RMSECV) and the value of the residual prediction deviation (RPD) of chocolates were compatible. NIRS prediction of CP, EE, CF, sugar, starch, NSC, P, Fe and Cu contents were more precise, with high R^2 (0.98, 0.95, 0.90, 0.99, 0.95, 0.95, 0.98, 0.98 and 0.94); low RMSECV (0.27, 1.28, 0.52, 0.02, 0.51, 1.21, 0.005, 3.31 and 3.62); and high RPD (8.6, 4.5, 3.72, 11.00, 5.19, 5.63, 7.52, 9.02 and 4.38 respectively). Satisfactory results in NIRS were also obtained for the DM, CA, Ca, K, Mg, and Na prediction. The equations for these parameters showed a good ability ($R^2 \geq 0.76$, RPD $\geq$ 1.57). For the other parameters such as OM, ADF, NDF, ADL, DMD, OMD, ME_{nc}, ME_e, Zn and Mn were observed poor validation results ($R^2 \leq 0.64$, RPD $\leq$ 0.44).

It was concluded that NIRS has a good the potential to provide fast nutritional diagnoses for using formal foodstuff chocolates as feed and can be used as a screening method for the control of the chemical composition, the energy, and the digestibility value of chocolates for ruminants.

Key words: *NIRS, chocolate, nutrient content, prediction*

---

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Ege University, Faculty of Agriculture, Department of Animal Science, İZMİR-TURKEY
[2] Adnan Menderes University, Faculty of Agriculture, Department of Animal Science, AYDIN-TURKEY
[*]Corresponding Author: Hayrullah Bora Ünlü, hayrullah.bora.unlu@ege.edu.tr

INTRODUCTION

The chemical composition and the energy value of feeds for ruminants vary considerably because of the wide choice in raw materials and by-products. Commercial feed producers, nutritionist or farmers need to know quality of the purchased concentrates, not only economic reasons but also from a feed-technical and environmental viewpoint. Nutrient supply to the individual requirements of the animals is essential to achieve their production potential and to avoid unnecessary mineral losses to the environment (De Boever *et al.*, 1995).

The potential of near infrared reflectance spectroscopy (NIRS) as a technique for rapidly estimating various composition measures of forages and raw materials has considerable research attention (Lippke and Barton 1988). The development of the NIRS technology during the last two decades has opened the new perspectives for a cheap, fast and accurate evaluation of organic materials. The wet chemical analyses of feed samples to determine their chemical composition are time consuming and expensive (Cheli *et al.,* 2012). Animal nutritionist, farmer and commercial feed producers require an accurate, precise, rapid and cost effective method of evaluating the nutritive value of forage, raw and by-products feed materials (Undersander, 2006). NIRS provides an opportunity to determine the chemical composition and digestibility of feedstuffs (Lippke and Barton 1988; De Boever *et al.*, 2003). Apart from its rapidity, NIRS is a physical non-destructive method, requiring minimal sample preparation with high accuracy, a low environmental impact and no harmful chemicals are used (Cheli *et al.,* 2012). In contrast to traditional chemical analyses, NIRS require no reagents, producing no waste. It is further more a multi- analytical techniques as several determinations can be made simultaneously and once the NIRS is calibrated, it is simple to use and operate (Givens *et al.*, 1997). For example; conventional chemical analyses of feeds will take two to three days, while a similar analysis can be completed in 2-3 minutes by NIRS (Corson *et al.*, 1999). Almost all of the research and use of NIRS technique for feed analyses started with the work Karl Norris on the determination of moisture in agricultural products by NIRS in 1965. Working principle of NIRS is that bonds between organic molecules absorb a specific wavelength range of light in the rear infrared region and the near infrared color of the samples provides information about its composition (Rahman *et al.,* 2015). A more limited number of publications concerning the use of NIRS with compound feedstuffs and by-products feeds were reported. This is because the considerable heterogeneity of these samples was supposed to require a great number of samples for calibrations (Perez-Marin *et al.,* 2004).

Animal production and animal feeds have changed significantly in the past 60 years. Small family owned and operated farms have been turned into a professional enterprise with large production capacity. This system has increased feed efficiency and animal production capacity in the new generation animals (Wilkinson, 2011) because it allows the production of genetically high yielding animals (Sapkota *et al.*, 2007). For this reason, the quality and quantity of feed provided to animals is the most important input for increasing the animal productivity (Alltech, 2015). Meanwhile, it is extremely important to use feed resources effectively and with minimum waste for the development of sustainable animal husbandry. Some feedstuffs in animal nutrition are also commonly used for human food and fuel oil production, and this reality limits the use of these feedstuffs in feed industry and in return increases their prices (Ajila *et al.*, 2012). Therefore, producers are looking for low-cost feedstuffs that will provide higher weight gain, and milk production with more intense rations in same dry matter for energy and protein (Theurer *et al.*, 1999).

Former foodstuffs that is one of the non-conventional feed resources are other than catering reflux which were manufactured for human consumption in full compliance with the EU food law but which are no longer intended for human consumption for practical or logistical reasons or due to problems of manufacturing or packaging defects or expiration of internal sell-by date, and which do not present any health risks when used as feed (EFFPA, 2017). Ruminants also have the high potential to examine these raw materials. Typical former foodstuffs used as animal feed are chocolate, biscuits, breads, breakfast cereals, pasta, and candies (Westendorf, 2000). Because of high energy content in the form of sugars, oils and starch, former feedstuffs can be replaced to cereals as an energy source certain rate and can also reduce the cost of ration. Chocolate, with valuable compounds that are rich energy, fat and sugar content as well as cocoa flavor and aroma, is potentially a good alternative feed material especially in ruminant feeding (Unlu *et al.*, 2017). Chocolate contains cocoa butter, sugar, cocoa, milk, milk powder, and aroma ingredients, and is a sweet and delicious food. However, such practices do not yet scientifically exist, and there is not enough information about the use of chocolate in ruminant nutrition. Also, simple analytical methods need to rapid and accurate feedstuff evaluation for by-products feeds or formal foodstuffs such as chocolates.

The aim of the present study was to evaluate the efficacy of NIRS in predicting the chemical compositions, energy values, mineral contents and digestibility of chocolates as a feed material between chemical methods and NIRS.

MATERIALS AND METHODS

Thirty six different chocolate samples were taken from six different production and storage units. All chocolates samples were analyzed for parameters by wet chemistry method (WCM). Experimental chocolate samples were ground through a 1 mm screen in preparation for chemical analysis. Crude matter (DM), crude ash (CA), ether extract (EE), crude protein (CP), crude fiber (CF) contents of samples were determined by the Weende analysis method (AOAC, 1995). Organic matter contents were calculated as OM=DM-CA. The simple or nonstructural carbohydrates (NSC) contents were calculated using the equation of 100-% (DM + CP + EE + CA + CF). Cell wall contents of samples as the neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed according to Van Soest (Goering and Van Soest, 1970). The sugar content of the materials was determined by the Luff-

Scroll method and the starch determination by polarimetric method. Phosphorus (P) contents of the materials were read by spectrophotometer (model PE General TU-1880 Model Double Beam UV-V15) by calorimetric methods. Atomic absorption spectroscopy (A400, PERKIN ELMER) was used for determining Ca, K, Mg, Na, Zn, Fe, Mn, Cu concentrations. Estimates for metabolic energy (ME_{nc}) as Kcal/kg in DM were based on protein, fiber and fat levels determined from the samples using a prediction equation (TSI-9610, 1991).

Dry matter digestibility *in vitro*, organic matter digestibility *in vitro* and ME_e were determined according to cellulaz technic method (De Boever *et al.*, 1986 modified from Tilley and Terry, 1963). Chemical analyses were applied in Ege University Faculty of Agriculture Department of Animal Science and Animal Feed Chemical Analysis Laboratory, İzmir. Chemical analyses were performed in duplicate.

NIRS analysis was done with an Bruker MPA (Multi-Purpose Analyzer; Bruker Optics, Germany) using OPUS spectroscopy software statistic version 7. The chemical analysis results of the thirty six chocolate samples loaded the on the NIRS and then each samples were scanned for calibration. The samples were ground through a 1 mm screen in preparation for NIRS. The samples were air- dry and each sample were scanned twice in closed cups and the two scans were averaged. The Bruker MPA instrument measures reflected light for chocolate samples in the range of 4000-12800 cm^{-1} at approximately 16 cm^{-1} interval. Calibrations were developed by means of partial least-squares (PLS) regression (OPUS/QUANT software, Bruker, Germany). PLS is the appropriate multivariate calibration technique to avoid the problem of the very high intercorrelation between absorbances (Goedhart, 1990). Calibration equations were evaluated in terms of determination (R_c^2) and the root mean square error of estimation (RMSEE) respectively. Validation equations were evaluated in terms of coefficient of determination (R_v^2) and the value of the root mean square error of cross validation (RMSECV). Another statistic used was RPD that the residual prediction deviation is the ratio of standard deviation to standard error of prediction.

RESULT AND DISCUSSION

The nutritive value of formal food stuffs chocolate indicated by a number of chemical parameters for ruminants. The chemical composition, digestibility and energy value of chocolates for ruminant are listed in Table 1. DM content (%) were obtained 96.24 by WCM, 97.76 by NIRS; CA content (%) were obtained 1.82 by WCM, 1.76 by NIRS; OM content (%) were obtained 96.64 by WCM, 95.95 by NIRS; EE content (%) were obtained 31.80 by WCM, 31.06 by NIRS; CP content (%) were obtained 7.99 for WCM, 7.74 by NIRS; CF content (%) were obtained 4.67 by WCM, 4.37 for NIRS; NSC content (%) were obtained 50.43 by WCM, 50.86 by NIRS; NDF content (%) were obtained 8.53 by WCM, 7.99 by NIRS; ADF content (%) were obtained 2.62 by WCM, 2.73 by NIRS; ADL content (%) were obtained 0.36 for WCM, 0.38 by NIRS; starch content (%) were obtained 6.84 by WCM, 6.25 for NIRS; sugar content (%) were obtained 23.79 by WCM, 23.24 by NIRS; DMD value (%) were obtained 83.87

by WCM, 83.71 by NIRS; OMD value (%) were obtained 84.31 by WCM, 83.00 by NIRS; ME_{nc} content (Kcal/kg) were obtained 4142 for WCM, 4050 by NIRS; ME_e content (Kcal/kg) were obtained 4060 by WCM, 4070 for NIRS. The findings about nutrition composition, digestibility and energy value of chocolates for ruminant are consistent with the findings of the relevant studies in general NRC (1989); NRC (2001) and Unlu *et al.*, (2017).

The chemical characteristics of the chocolates samples extended a large range variation. The all parameters of chemical composition, in vivo enzymatic digestibility and energy value of chocolate samples also showed a considerable range, which could be expected from the variable ingredient composition for producing to chocolate. The variation of the chemical composition parameters of the chocolate may be based on the differences between chocolate types or the fact that the chocolate is mixed with candy-biscuits. Chemical characteristics of byproduct feeds and former foodstuffs are very variable, owing to different modes of production processing, consumption habits, and/or the mixing of different types of chocolates (Unlu *et al.*, 2017).

The statistics of NIRS calibrations and validations for chemical and nutritive characteristics are listed in Table 2. The calibration and validation sets showed a comparable distribution for all variables. The coefficient of determination for EE, CP, CF, NSC, starch and sugar showed an excellent ability in both calibration (R_c^2) 0.99, 0.99, 0.99, 0.99, 0.99, 0.99 and validation (R_v^2) 0.95, 0.98, 0.90, 0.95, 0.95, 0.99 with high RPD_c 57.7, 52.0, 38.1, 13.4, 244, 21.6 and high RPD_v 4.5, 8.6, 3.7, 5.6, 5.1, 11.0 respectively. The RPD should ideally be at least three, although the variance in reference data is low (Williams and Sobering, 1996). The strong absorption of fat and protein in the NIRS region is well known (Shenk *et al.*, 1992). Therefore, the high coefficient of determination for EE and CP are not surprising (Table 2). Also RMSEE 0.14, 0.05, 0.05, 0.73, 0.01, 0.01 and RMSCEV 1.28, 0.27, 0.52, 1.21, 0.51, 0.02 were found low respectively. The high R^2 and RPD values for EE, CP, CF, NSC, starch and sugar were not only due to the clear absorbance of these compositions in the NIRS but also to the good reproducibility of the reference method. The findings about EE, CP, CF, of chocolates are consistent with the findings of the relevant studies in general, Xiccato *et al.*, (2003) for rabbit feeds, Perez Martin *et al.*, (2004) for compound feed stuffs, Edney *et al.*, (1994) for barley and Towett *et al.*, (2013).

DM and CA had good coefficient determinations for calibration (R_c^2) 0.97, 0.97 and validation (R_v^2) 0.81, 0.84 respectively and were relatively low as were also RPD_v values (1.57, 2.75), with RMSCEV (1.61, 0.16). The findings about DM of chocolates are consistent with the findings of the relevant studies in general (Xiccato *et al.*, 2003; Perez Martin *et al.*, 2004).

The OM, NDF, ADF, ADL, DMD, OMD, ME_{nc} and ME_e of calibrations had higher coefficients of determination than coefficients of determination prediction in DM. Also in both calibration (R_c^2) 0.51, 0.82, 0.67, 0.99, 0.62, 0.74, 0.58, 0.95 and validation (R_v^2) 0.40, 0.51, 0.59, 0.69, 0.64, 0.77, 0.56, 0.58 values of these parameters were poorly predicted relatively other parameters (Table 2). RMSCEV and RPD values of these parameters were low. The low NIRS prediction accuracy for OM, NDF, ADF, ADL, DMD, OMD, ME_{nc} and ME_e are not only due to low signal in the NIR region but also to the poor reproducibility of the reference method as demonstrated in previous studies (Xiccato *et al.,* 2003). Except the DMD and OMD coefficient of determination values of the chocolates, the findings about R_v^2, RPD_v and RMSCEV parameters of chocolates are consistent with the findings of the relevant studies in general (Landau *et al.,* 2006). The low coefficient values of these parameters may be due to low number of samples. For these parameters may be need to more than thirty six chocolate samples for good prediction accuracy. These findings in this study were compatible with De Boever *et al.,* (1995); Xiccato *et al.,* (1999) and Xiccato *et al.,* (2003).

The mean, range and standard deviation of the minerals measured by wet chemical method and NIRS are shown in Table 3. Ca content (%) were obtained 0.24 by chemical method, 0.22 by NIRS; P content (%) were obtained 0.17 by chemical method, 0.18 by NIRS; K content (%) were found 0.69 by chemical method, 0.67 by NIRS; Mg content (%) were found 0.16 by WCM, 0.12 by NIRS; Na content (%) were found 0.14 for WCM, 0.11 by NIRS; Zn content (mg) were found 18.75 by WCM, 10.12 for NIRS; Fe content (mg) were found 89.18 by WCM, 86.55 by NIRS; Mn content (mg) were found 8.76 by WCM, 8.89 by NIRS; Cu content (mg) were found 49.46 by WCM, 45.76 by NIRS. Ca, P, K, Mg, Na, Zn, Fe, Mn and Cu minerals of chocolates showed a wide range in composition due to different types of chocolate. The mean mineral values of obtained in this study were consistent with the findings of relevant studies (Unlu *et al.,* 2017). Because of this, the means found are considered to lie within the normal ranges for chocolates.

The statistics of NIRS calibrations and validations for chemical mineral contents of chocolates are listed in Table 4. Coefficient of determination in validation (R_v^2), low standard error of prediction (RMSCEV) and high residual prediction deviation (RPD) were excellent for P, Fe and Cu. These values of P, Fe and Cu were obtained for R_v^2 0.98, 0.98, 0.93; for RMSCEV 0.005 %, 3.31 mg, 3.62 mg; for RPD 7.52 %, 9.02 mg, 4.38 mg respectively. Coefficient of determination in validation (0.79, 0.76, 0.79 and 0.78) standard error of prediction (0.027 %, 0.103 %, 0.020 % and 0.012 %) and residual prediction deviation (2.85 %, 2.05 %, 2.38 % and 2.41 %) were satisfactory for Ca, K, Mg and Cu respectively. But Zn and Mn minerals of chocolates had poor prediction accuracy with low R_v^2 0.40, 0.45; RMSCEV 3.58 mg, 3.62 mg and low RPD 1.30 mg, 1.48 mg respectively. The prediction for Zn and Mn were poor because minerals do not absorb in the near infrared region, which corresponds with results reported by De Boever *et al.,* (1995) for compound feeds for cattle; Cozzolino and Moron (2004) for minerals in legumes. The coefficient of correlation, R^2, indicates the closeness of fit between the NIRS reflectance and reference data over the range of composition (Swart *et al.,* 2012). A high *r* value with a low SEP and bias, together with a slope close to 1.0, means that the NIRS reflectance results are accurate over the anticipated range and likely to remain so, provided that these statistics were based on a sufficient number of observations (Williams, 2001).

Table 1. Chemical composition and nutritive value of chocolate, dry matter basis (%)

%	WCM Minimum	WCM Maximum	WCM Mean±SD	NIRS Minimum	NIRS Maximum	NIRS Mean±SD
Dry Matter	92.65	98.80	96.24±1.84	93.48	99.29	97.76±1.87
Crude Ash	1.25	2.29	1.82±0.25	1.04	2.25	1.76±0.36
Organic Matter	93.68	98.75	96.64±1.50	93.84	97.28	95.95±1.17
Ether Extract	20.50	39.81	31.80±5.13	23.30	38.16	31.06±5.27
Crude Protein	5.58	11.64	7.99±1.33	4.89	9.81	7.74±1.48
Crude Fiber	2.04	7.59	4.67±1.67	2.00	7.89	4.37±1.88
NSC	42.97	62.80	50.43±3.91	40.51	64.08	50.86±6.70
NDF	5.70	11.59	8.53±1.40	6.00	12.12	7.99±2.01
ADF	1.20	3.74	2.62±0.48	2.07	3.46	2.73±0.48
ADL	0.20	0.98	0.36±0.17	0.13	0.92	0.38±0.26
Starch	2.20	9.97	6.84±1.89	2.36	10.84	6.25±2.84
Sugar	22.99	24.62	23.79±0.36	22.17	23.56	23.24±0.33
DMD	74.75	91.03	83.87±5.37	78.49	91.47	83.71±4.05
OMD	73.79	95.69	84.01±6.53	73.16	96.78	83.00±7.98
ME_{nc} Kcal/kg	3866	4505	4142±227	3551	4747	4050±265
ME_e Kcal/kg	3900	4658	4060±178	3752	4610	4070±272

Table 2. Coefficients of determination and calibration (R_c^2) and validation (R_v^2), RMSEE, RMSCEV, and RPD index obtained by PLSR to predict the chemical composition and the nutritive value in chocolates

	Calibration (n=36)			Validation (n=36)		
	R^2	RMSEE	RPD	R^2	RMSCEV	RPD
Dry Matter	0.97	0.39	6.57	0.81	1.61	1.57
Crude Ash	0.97	0.07	6.24	0.84	0.16	2.75
Organic Matter	0.51	1.52	1.44	0.40	2.12	0.85
Ether Extract	0.99	0.14	57.7	0.95	1.28	4.50
Crude Protein	0.99	0.05	52.00	0.98	0.27	8.60
Crude Fiber	0.99	0.05	38.10	0.90	0.52	3.72
NSC	0.99	0.73	13.4	0.95	1.21	5.63
NDF	0.82	0.91	2.39	0.51	1.36	1.46
ADF	0.67	0.43	1.76	0.59	0.61	1.12
ADL	0.99	0.04	10.8	0.69	0.17	2.14
Strach	0.99	0.01	244	0.95	0.51	5.19
Sugar	0.99	0.01	21.6	0.99	0.02	11.00
DMD	0.99	5.62	0.31	0.64	7.86	0.44
OMD	0.74	6.00	1.97	0.77	7.60	1.14
ME_{nc} Kcal/kg	0.58	207	1.56	0.56	210	1.25
ME_e Kcal/kg	0.95	0.36	4.61	0.58	0.93	1.39

Table 3. Mineral composition of the chocolates, dry matter basis (%)

	WCM			NIRS		
	Minimum	Maximum	Mean± S.D.	Minimum	Maximum	Mean ± S.D.
Ca, %	0.18	0.35	0.24±0.03	0.11	0.29	0.22±0.04
P,%	0.12	0.24	0.17±0.02	0.13	0.21	0.18±0.02
K,%	0.45	0.91	0.69±0.14	0.32	0.88	0.67±0.18
Mg,%	0.06	0.16	0.11±0.02	0.06	0.18	0.12±0.03
Na,%	0.07	0.90	0.14±0.13	0.08	0.17	0.11±0.02
Zn, mg	9.33	23.85	18.75±2.05	5.31	17.85	10.12±3.73
Fe,mg	55.68	135.15	89.18±23.67	25.05	130.78	86.55±36.62
Mn,mg	4.89	12.36	8.76±1.54	5.48	12.15	8.89±2.18
Cu, mg	31.30	67.75	49.46±11.72	23.68	63.16	45.76±12.76

Table 4. Coefficients of determination and calibration (R_c^2) and validation (R_v^2), RMSEE, RMSCEV, and RPD index obtained by PLSR to predict the mineral compositions in chocolates

	Calibration (n=36)			Validation (n=36)		
	R^2	RMSEE	RPD	R^2	RMSCEV	RPD
Ca, %	0.99	0.001	16.8	0.79	0.027	2.85
P,%	0.99	0.001	66.1	0.98	0.005	7.52
K,%	0.87	0.084	2.87	0.76	0.103	2.05
Mg,%	0.97	0.008	6.26	0.79	0.020	2.38
Na,%	0.99	0.001	58.5	0.78	0.012	2.41
Zn, mg	0.90	1.71	3.25	0.40	3.58	1.30
Fe,mg	0.99	0.08	7.92	0.98	3.31	9.02
Mn,mg	0.98	0.39	8.26	0.45	1.88	1.48
Cu, mg	0.99	1.70	10.4	0.93	3.62	4.38

CONCLUSION

In this study, it seems that NIRS calibration are successfully for enough predicting ether extracts, crude protein, crude fiber, nonstructural carbohydrates, starch, sugar, iron and copper content of formal foodstuffs chocolate for ruminant nutrition. Also NIRS showed a good potential of to predict dry matter, crude ash, organic matter digestibility, calcium, potassium, magnesium and sodium. Other parameters need to much more calibration samples for good prediction.

NIRS technology enables rapid and ecologic compliance with rules relating to the evaluating of feedstuffs, avoiding the need for using reagents and production of chemical residues. NIRS must therefore be considered as an essential method for sustainable animal production. In conclusion, this research showed that NIRS can be used successfully for predicting nutritional composition of formal foodstuffs too.

REFERENCES

Ajila, C. M., Brar, S. K., Verma, M., Tyagi, R., Godbout, D. S., Valéro, J. R. (2012): Bio-processing of agro-byproducts to animal feed, Critical Reviews in Biotechnology, 32(4):382–400.

Alltech, (2015): Global feed survey. ALLTECH, 3031 Catnip Hill Road, Nicholasville, Kentucky 40356, USA, 01.03.2017, http://www.alltech.com/sites/default/files/global-feed-survey-2015.pdf

Association of Official Analytical Chemists (AOAC), (1995): Official methods of analysis, 16th Edition. Edited by Patricia Cunniff., Virginia, USA.

Cheli, F., Battaglia, D., Pinotti, L., Baldi, A. (2012): State of the art in feedstuff analysis: A technique-oriented perspective. J. Agric. Food Chem., 60:9529−9542.

Corson, D. C., Waghom, M. J., Ulyatt, M. J., Lee, J. (1999): NIRS: Forage analysis and livestock feeding. Proceedings of New Zealand Grassland Association, 61:127-132.

Cozzolino, D., Moron, A. (2004): Exploring the use of near infrared reflectance spectroscopy (NIRS) to predict trace minerals in legumes. Animal Feed Science and Technology, 111:161–173.

Edney, M. J., Morgan, J. E., Williams, P. C., Campbell, L. D. (1994): Analysis of feed barley by near infrared reflectance technology. *J. Near Infrared Spectrosc.*, 2:33–41.

EFFPA,. (2017): European Former Foodstuff Processors Association, 02.01.2017, http://www.effpa.eu.

De Boever, J. L., Cottyn, B. G., Buysse, F. X., Wainman, F. W., Vanacker, J. M. (1986): The use of an enzymatic technique to predict digestibility, metabolizable energy of compound feedstuffs for ruminants, Animal Feed Science and Technology, 14:203-214.

De Boever, J. L., Cottyn, B. G., Vanacker, J. M., Boucqué, C. V. (1994): The use of NIRS to predict the chemical composition and the energy value of compound feeds for cattle, Anim. Feed Sci. Technol., 51:243-253.

Givens, D. I., De Boever, J. L., Deaville, E. R. (1997): The principles, practices and some future applications of near infrared spectroscopy for predicting the nutritive value of foods for animals and humans, Nutrition Research Reviews, 10:83-1 14.

Goedhart, P. W. (1990): Comparison of multivariate calibration methods for prediction of feeding value by near-infrared reflectance spectroscopy. Neth. J. Agric. Sci., 38:449-460.

Goering, H. K., Van Soest, P. J. (1970): Forage fiber analyses, agriculture handbook, Washington D. C., p: 379.

Landau, S., Glasser, T., Dvash, L. (2006): Monitoring nutrition in small ruminants with the aid of near infrared reflectance spectroscopy (NIRS) technology: A review. Small Ruminant Research, 61: 1–11.

Lippke, H., Barton F. E. (1988): Near infrared reflectance spectroscopy for predicting intake of digestible organic matter by cattle**,** J. Dairy Sci., 71:2986-2991.

Norris, K. H. (1965): Measuring and using light transmittance properties of plant materials, in: Electromagnetic Radiation in Agriculture, Illuminating Engineering Soc. and ASAE, New York.

NRC, (1989): Nutrition requirement of beef cattle. National Academic Press, Washington, D. C., ISBN Number:0-309-06934-3, p:232.

NRC, (2001): Nutrition requirement of dairy cattle. National Academic Press, Washington, D. C., ISBN:0-309-06997-1, p:381.

Pérez-Mar´ın, D. C., Garrido-Varo, A., Guerrero-Ginel, J. E., Gómez-Cabrera, A. (2004): Near-infrared reflectance spectroscopy (NIRS) for the mandatory labeling of compound feeding stuffs: chemical composition and open-declaration. Animal Feed Science and Technology, 116:333–349.

Rahman, A., Bayram, I., Khanum, S., Ullah, S. (2015): Use and calibration of near infrared reflectance spectroscopy in feed analysis: A mini review. Pak. J. life Soc. Sci., 13(l):1-7.

Sapkota, A. R., Lefferts, L. Y., McKenzie, S., Walker, P. (2007): What do we feed to food-production animals? A review of animal feed ingredients and their potential impacts on human health. Environmental Health Perspectives, 115(5):663-670.

Shenk, J. S., Workman, J. J., Westerhaus, M. O. (1992): Application of NIR spectroscopy to agricultural products. In: Burns, D. A., Ciurczak, E. W. (Eds.), Handbook of Near-Infrared Analysis. Marcel Dekker, New York, p:383–431.

Swart, E., Brand, T. S., Engelbrecht, J. (2012): The use of near infrared spectroscopy (NIRS) to predict the chemical composition of feed samples used in ostrich total mixed rations, South African Journal of Animal Science, 42(5), Supplement 1: 550-554.

Theurer, C. B., Huber, J. T., Delgado-Elorduy, A., Wanderley, R. (1999): Invited review: Summary of steam-flaking corn or sorghum grain for lactating dairy cows, Journal of Dairy Science, 82: 1950-1959.

Tilley, J. M. A., Terry, R. A. (1963): A two stage technique for the in vitro digestion of forage crops, Journal of The British Garssland Society, 18:104-111.

Towett, E. K., Alex, M., Keith, D., Shepherd, K. D., Polreich, S., Aynekulu, E., Maass, B. L. (2013): Applicability of near-infrared reflectance spectroscopy (NIRS) for determination of crude protein content in cowpea (Vigna unguiculata) leaves. Food Science & Nutrition 3, 1(1):45–53.

TSI, (1991): Turkish Standard Institute, Animal feeds-metabolizable energy determination (chemical method). TSI number: 9610, Ankara, Turkey, [in Turkish].

Undersander, D. (2006): Uses and abuses of NIR for feed analysis. 17th Annual Meeting, February 1-2, 2006 Florida Ruminant Nutrition Symposium, Best

Western Gateway Grand, Gainesville FL. 15.08.2017, http://dairy.ifas.ufl.edu/rns/2006/Undersander.pdf.

Unlu, H. B., Ozelcam, H., Kirkpinar, F., Ozdogan, M. (2017): A sweet alternative for ruminants: chocolate. 2nd International Balkan Agriculture Congress, 16-18 May 2017, Tekirdağ/Turkey, ISBN:978-605-4265-46-6, 418.

Westendorf, M. L. (2000): Food waste to animal feed. Iowa State University Press, Ames-Iowa, ISBN: 978-0-8138-2540-3, USA:249-257.

Wilkinson, J. M. (2011): Re-defining efficieny of feed use by livestock. Animal 5:7, p:1014-1022.

Williams, P. C., Sobering D. (1996): How do we do it: a brief summary of the methods we use in developing near infrared calibrations. In: Davies, A. M. C.,Williams, P. C. (Eds.), Near Infrared Spectroscopy: The Future Waves. NIR Publications, Chichester, West Sussex, UK, 185–188.

Williams, P. C. (2001): Implementation of near-infrared technology. In: Near-infrared Technology in the Agricultural and Food Industries, 2nd ed. Eds Williams, P. & Norris, K., St. Paul, USA: American Association of Cereal Chemists., 145-169.

Xiccato, G., Trocino, A., Carazzolo, A., Meurens, M., Maertens, L., Carabaño, R. (1999): Nutritive evaluation and ingredient prediction of compound feeds for rabbits by near-infrared reflectance spectroscopy (NIRS), Anim. Feed Sci. Technol., 77:201–212.

Xiccato, G., Trocino, A., De Boever, J. L., Maertens, L., Carabaño, R., Pascual, J. J., Pérez, J. M., Gidenne, T., Falcao-E-Cunha, L. (2003): Prediction of chemical composition, nutritive value and ingredient composition of European compound feeds for rabbits by near infrared reflectance spectroscopy, Anim. Feed Sci. Technol., 104:153–168.

BLOOD REFERENCE VALUES OF CERTAIN BIOCHEMICAL PARAMETERS IN MERINOLANDSCHAF LAMBS FROM ORGANIC FARMING*

Zvonko Antunović[1], Marcela Šperanda[1], Željka Klir[1], Krunoslav Zmaić[1], Mislav Djidara[1], Josip Novoselec[1]

Original scientific paper

Summary

The aim of the present research was to determine reference values of certain biochemical parameters in blood of 40 Merinolandschaf lambs in organic farming. After weaning lambs were fed with feed mixture and meadow hay of organic origin *ad libitum*. Average body mass of lambs was 34.01 kg, and average body condition score was 3.86. Within blood serum the concentrations of mineral parameters (calcium, phosphorus-inorganic, magnesium and iron), concentrations of the biochemical parameters (urea, glucose, total proteins, albumin, cholesterol, HDL-cholesterol; LDL-cholesterol, triglyceride, BHBA-β-hydroxibutyrate and NEFA-non-esterified fatty acids) and enzyme activity (CK-creatine kinase, ALP-alkaline phosphatase) were examined. Most of the biochemical parameters´ concentrations in the lambs´ blood was within reference values for lambs, except albumin (28.05 g/L), Ca and Mg (2.43 and 0.90 mmol/L) concentrations as well as activity of CK enzyme (170.49 U/L) which was bellow or on the lower reference values as well as concentration of globulins (30.02 g/L) which was higher compared to reference values. Determined changes of biochemical parameters in the blood of Merinolandschaf lambs in organic farming indicated the need for redefinition of these parameters as reference values and presents adequate supply of lambs with nutrients through diets. Determined biochemical parameters in the blood of Merinolandschaf lambs in organic production can be used as reliable criteria in assessment of nutrients supply through diets, as well as redefinition of reference values´ limits of these parameters in the blood, as well as their reference values.

Key words: *lambs, blood, biochemical parameters, Merinolandschaf, organic farming*

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Faculty of Agriculture in Osijek, J. J. Strossmayer University in Osijek
Correspondence: prof. dr. sc. Zvonko Antunović, V. Preloga 1, 31000 Osijek, Croatia, zantunovic@pfos.hr

Rezime

Cilj ovoga rada bio je utvrditi referentne vrijednosti određenih biokemijskih pokazatelja u krvi 40 Merinolandschaf janjadi u ekološkim uzgojima. Janjad je nakon odbića hranjena krmnom smjesom i livadnim sijenom ekološkog podrijetla po volji. Prosječna tjelesna masa janjadi bila je 34,01 kg, a prosječan indeks tjelesne kondicije 3,86. U serumu su utvrđene koncentracije minerala (kalcij, fosfor-anorganski, magnezij i željezo), biokemijskih pokazatelja (urea, glukoza, ukupne bjelančevine, albumin, kolesterol, HDL-kolesterol; LDL-kolesterol, trigliceridi, BHBA-β-hidroksibutirat i NEFA-neesterificirane masne kiseline) i aktivnost enzima (CK-kreatin kinaza, ALP-alkalna fosfataza). Većina koncentracija biokemijskih pokazatelja u krvi janjadi bila je u granicama referentnih vrijednosti za janjad, osim koncentracija albumina (28,05 g/L), Ca i Mg (2,43 i 0,90 mmol/L) te aktivnosti CK (170,49 U/L) koje su bile ispod ili na donjoj granici referentnih vrijednosti te koncentracije globulina (30,02 g/L) koje su bile iznad referentnih vrijednosti. Utvrđene promjene biokemijskih pokazatelja u krvi Merinolandschaf janjadi u ekološkom uzgoju ukazuju na potrebu redefiniranja navedenih pokazatelja kao referentne vrijednosti te pokazuju zadovoljavajuću opskrbljenost janjadi hranjivim tvarima obrokom. Utvrđeni biokemijski pokazatelji u krvi Merinoladschaf janjadi u ekološkom uzgoju mogu se koristiti kao pouzdan kriterij u procjeni opskrbljenosti janjadi hranjivim tvarima putem obroka te, uz redefiniranje graničnih referentnih vrijednosti navedenih pokazatelja u krvi, kao i njihove referentne vrijednosti.

Ključne riječi: *janjad, krv, biokemijski pokazatelji, Merinolandschaf, ekološki uzgoj*

INTRODUCTION

Sheep production in the Republic of Croatia is based on rearing of Croatian native breeds of sheep which are adapted on the environmental conditions of the particular area of sheeps´ origin. According to unique register of domestic animals in the Republic of Croatia on day 31st of December 2016, 632.087 sheep at 19.249 farms were reared (CAA, 2017.). Dalmatinska pramenka is the most dominant breed, and among the foreign breeds of sheep is Merinolandschaf (Antunović *et al*., 2012). Local breeds have economic values as well as social, cultural and environmental (Bojkovski *et al*., 2015). Rearing of Merinolandschaf breed in our region is successful for many years. Good quality farming of Merinolandschaf sheep is located in the continental part of our country (Slavonia and Baranja, Podravina, Bilogora, Posavina), and a smaller number of them are within organic production. According to the Croatian Agricultural Agency (CAA) in the Republic of Croatia in year 2016 from total number of sheep in herdbook, which were accompanied by selection work (39.122 heads in 397 sheep breeders), a significant proportion was related to Merinolandschaf 4.46% (1743 heads in 18 sheep breeders). The average size of flocks in Merinolandschaf sheep was 97 animals. Analyzing reproductive indicators it is evident that the index of lambing in Merinolandschaf sheep was 1.00, and the litter size 1.13, while the lambs in the field test were the average birth weight of 4.43 kg, average daily gain of 388 g, and the final body weight of 46.44 kg. According to CAA (2017) most of the lambings were in February (43.79%), January (26.63%) and March (16.57%).

Concentrations of blood biochemical parameters in animal depend on several factors (age, sex, stress, diet, production, handling, climate, physiological status, laboratory methodology and breed; Kaneko *et al*., 2008; Tschuor et al. 2008; Antunović *et al*., 2002, 2004 and 2005; Schweinzer *et al.*, 2017; Shek Vugrovečki *et al.*, 2017). Considering the number of Merinolandschaf sheep it is necessary to monitor production and feeding (Antunović *et al*., 2010a, b) as well as establish their reference values for individual biochemical parameters in the blood, in addition to those related to the monitoring of their energy, protein and nutritional status as well as mineral status of sheep and lambs in organic farming. Hrković *et al*. (2009) concluded that the importance of studying the blood parameters enhances the knowledge and interaction of local farming and sheep metabolism in the function of the sustainability of original breeds.

The aim of the present paper was to determine the reference values of certain biochemical parameters in the blood of Merinolandschaf lambs in organic farming.

MATERIAL AND METHODS

The research was conducted on the organic family farm in Osijek-Baranja county. In the present study 40 lambs of Merinolandschaf were included, one month after weaning (age of 120 days). After weaning lambs were fed with feed mixture (17% of crude protein) and meadow hay of organic origin *ad libitum*. Rearing and feeding of lambs were according to Council Regulation (EC, 2007). Average body weight of lambs was 34.01 kg, and average body condition score, determined according to Russel (1991) was 3.86.

The blood was collected from the jugular vein (10mL) into the sterile vacuum tubes Venoject® (Sterile Terumo Europe, Leuven, Belgium), containing both Li-heparin as anticoagulants. After that, serum was separated by centrifugation (10 min) at 3000 revolutions/min and placed into the Olympus AU400. Within blood serum the concentrations of the mineral parameters (calcium, phosphorus-inorganic, magnesium and iron) were found, concentrations of the biochemical parameters (urea, glucose, total proteins, albumin, cholesterol, HDL-cholesterol; LDL-cholesterol, triglyceride, BHBA-β-hydroxibutyrate and NEFA-non-esterified fatty acids) and enzyme activity (CK-creatine kinase, ALP-alkaline phosphatase) with Olympus System Reagents (OSR), manufactured and distributed by Olympus Diagnostic GmbH (Irish Branch), Lismeehan, Ireland. Globulin content was calculated as the difference between total protein and albumin. Ratio albumin/globulin (A/G) was calculated as the difference between albumin and globulin. The analytical method of each parameter and the corresponding quality laboratory assay are reported in Table 1.

Table 1. Analytical methods for blood parameters and quality laboratory assays

Analytical method	Intra-assay variation (CV%)	Inter-assay variation (CV%)
Enzymatic Colorimetric method test for the quantitative determination of NEFA	4.81	4.32
Enzymatic method test for the quantitative determination of D-3 hydroxybutyrate	3.78	5.25
Photometric colour test for the quantitative determination of albumin	1.17	1.55
Enzymatic colour test for the quantitative determination of cholesterol	0.72	1.45
Enzymatic colour test for the quantitative determination of HDL-cholesterol	0.85	1.92
Enzymatic UV test (hexokinase method) for the quantitative determination of glucose	0.54	0.97
Photometric colour test for the quantitative determination of total protein	0.51	0.70
Enzymatic colour test for the quantitative determination of triglyceride	0.72	1.03
Kinetic UV test for the quantitative determination of urea	1.1	1.93
Kinetic colour test for the quantitative determination of alkaline phosphatase, EC 3.1.3.1 (ALP)	0.56	0.99
Kinetic UV test for the quantitative determination of creatine kinase, EC 2.7.3.2 (CK)	1.00	3.20
Photometric colour test for the quantitative determination of total calcium	0.65	0.95
Photometric UV test for the quantitative determination of inorganic phosphorous	0.63	1.33
Photometric colour test for the quantitative determination of iron	0.66	1.77
Photometric colour test for the quantitative determination of magnesium	0.75	1.29

Determined results were analyzed with statistical program SAS (SAS, 2008.). Average mean values and measures of variability were obtained with MEANS procedure.

RESULTS AND DISCUSSION

Adequate energy supply is the most important metabolism parameters in assessing the feeding status and health status determination. In addition to the determination of body condition of the investigated animals, which is a traditional way for determination of their energy status, concentration of non-esterified fatty acids (NEFA), beta hydroxybutyrate acid (BHBA), glucose, cholesterol and triglycerides determination in the blood (Van Saun, 2006; Hatfield *et al*., 1999) can be also used. Van Saun (2000) pointed out that concentration of NEFA in serum is more sensitive to energy balance in animals. Fernandez *et al*. (2007) obtained that the plasma concentration of NEFA presents the nearest relation with the intake of metabolizable energy, being capable in itself of indicating the goat's energy status. Good supply of lambs with energy is shown in Table 2, where all determined parameters were within the reference values for lambs (Lepherd *et al*., 2009; Antunović *et al*., 2010c). This statement is in accordance with good body condition score (3.86) values. Determined lower values for concentrations of cholesterol, HDL cholesterol and LDL cholesterol compared to reference values for lambs may be related to the different age of lambs at blood sampling since increasing its concentration in blood increases with age. Similar results were found in the Morada Nova sheep by Carlos *et al*. (2015). Analyzing the blood parameters of lambs in organic farming, which are good indication of energy supply, it is apparent that they are comparable to other studies carried out in the organic farming of Merinolandschaf lambs (Antunović *et al*., 2009 and 2010c).

Table 2. Blood parameters in assessment of energy status of Merinolandschaf lambs in organic farming

Parameter, mmol/L	Mean	Min.	Max.	Sd	SEM	Ref. values*	N. of lambs outside reference values	
							Higher	Lower
Glucose	4.33	0.60	5.19	0.77	0.12	2.7-4.8	11	-
Cholesterol	1.16	0.60	1.57	0.21	0.03	1.04-1.67[1]	-	3
HDL-cholesterol	0.83	0.60	0.93	0.10	0.02	0.68-0.97[1]	-	4
LDL-cholesterol	0.16	0.01	0.45	0.11	0.02	0.10-0.50[1]	-	3
Triglyceride	0.47	0.35	0.69	0.07	0.02	0.20-0.50[1]	12	-
NEFA	0.05	0.02	0.43	0.06	0.01	0.05[2]	6	27
BHBA	0.45	0.20	0.93	0.14	0.02	0.2-0.7	2	-

Sd-standard deviation, SEM-standard error mean; *Lepherd *et al*. (2009), [1]Antunović *et al*. (2010c); [2]Kiani (2013); NEFA-non-esterified fatty acids, BHBA-β-hydroxibutyrate.

Assessment of animal protein supply is more demanding and difficult process in relation to the assessment of energy status (Van Saun, 2000). Therefore, a combination of different blood parameters is used, including determination of urea, creatine, total protein, albumin and creatine kinase activity. However, there may also be a problem of proper interpretation of the results obtained due to various factors. For example, the urea concentration in the blood is influenced by mutually related factors such as the amount, amino acid composition and digestibility of proteins from feed, liver and kidney function, muscle tissue decomposition and the amount of carbohydrate in feed. Kohn *et al*. (2005) indicated that urea concentration in the blood may be very good indicator of the amount of nitrogen intake through feed.

Table 3. Blood parameters of protein status in Merinolandschaf lambs in organic farming

Parameter, g/L	Mean	Min.	Max.	Sd	SEM	Ref. values*	N. of lambs outside reference values	
							Higher	Lower
Urea, mmol/L	6.26	9.92	9.04	1.25	0.20	5.0-9.1	-	4
Total protein	59.06	45.20	71.50	4.65	0.74	51-64	4	1
Albumins	28.04	23.80	31.20	1.72	0.28	30-37	-	38
Globulins	30.02	21.40	41.80	4.66	0.64	19-30	23	-
A/G ratio	0.92	0.68	1.19	0.13	0.02	0.9-1.8	-	17
CK, U/L	170.49	84	448	82.29	13.01	180-454	5	33

*Lepherd *et al*. (2009); A-albumin, G-globulin, CK-creatine kinase

Determined concentrations of urea and total protein in the blood of lambs (6.26 mmol/L and 59.06 g/L) was within reference values by Lepherd *et al*. (2009). However, determined concentrations of albumin and activity of creatine kinase enzyme were slightly lower, and concentrations of globulin slightly above recommended reference values (Table 3). The above indicates the complexity of determining the supply of lambs with protein from diets as well as the satisfactory supply of lambs with proteins. Similar results determined Antunović *et al*. (2009, 2010c) in lamb of Merinolandschaf lambs. Considering determined differences related to concentrations of albumin and globulin compared to reference values it is necessary to make electrophoretic separation of protein fraction with aim for better understanding of the changes occurring in these parameters. Carlos *et al*. (2015.) determined similar values in the blood of Morada Nova sheep. In addition, these conclusions were also mentioned by Fayos *et al.* (2005). They concluded that animal age is one of important factors that may affect the concentrations of different serum protein fraction or their electrophoretic pattern. Although, many studies have been carried out to determine the usefulness of the determination of serum proteins and their electrophoretic pattern in various disease conditions and disorders in animals, serum protein evaluation is still a relatively little used diagnostic tool in veterinary medicine (Tothova *et al.*, 2016).

In numerous research (Bickhardt *et al.*, 1999; Roubies *et al.*, 2006) established reference values for the CK enzyme activity in liver blood is often much lower and fairly variable compared to the activity obtained in the studies of Lepherd *et al.* (2009) which presents a reference value. The authors pointed out that higher activity of CK enzyme in liver blood is determined as a result of a different blood sampling, which indicates the need to correct this parameter in Merinolandschaf lamb blood when determining reference values.

The estimation of mineral supply through animal nutrition is highly variable. Most of the minerals in the animal organism are tightly regulated by homeostatic mechanism and mostly the concentration of minerals (especially macroelements) in the blood is not taken as a reflection of nutrition when the homeostatic system is adequate. Due to minimal invasive process for the animal, blood sampling for mineral analysis is often applied, but it is not entirely successful (Kincaid, 2000). Concentrations of phosphorus, potassium, magnesium and sulfur in the blood of animals are highly sensitive depending upon nutrition. Calcium concentrations, except in the two weeks prior and after the partus, are not a reliable indicator of the supply of this feed element due to homeostatic regulation. The concentration of phosphorus in feed can also affect blood calcium concentrations because free phosphorus is involved in vitamin D synthesis. The short-term lack of microelements in nutrition of animals firstly causes activation of accumulated amounts so that vital biochemical functions are not reduced. Only their long-term lack in nutrition, when storage quantities are consumed, is visible in the form of lower concentrations in the transport part. If the nutritional deficiencies in feed are significant and long-lasting, all accumulated reserves will be empty, which will lead to occurrence of deficiency diseases (Herdt and Hoff, 2011). By analyzing the blood parameters (Table 4), the average Ca and Mg concentrations of lambs in organic farming were at the lower limit of the reference values, which may be related to the age of lambs. Fe and P-inorganic blood concentrations were within physiological limits for lambs. Similar concentrations for Ca, P-inorganic, Fe and ALP activity determined Antunovic *et al.* (2009, 2010b) in the blood of Merinolandschaf lambs in organic farming.

Table 4. Blood parameters of mineral status in Merinolandschaf lambs in organic farming

Parameter, mmol/L	Mean	Min.	Max.	Sd	SEM	Ref. values*	N. of lambs outside reference values	
							Higher	Lower
Ca	2.43	1.97	2.71	0.17	0.03	2.45-2.92	-	16
P-inoragnic	2.57	1.68	3.54	0.39	0.06	1.82-3.54	-	1
Mg	0.90	0.72	1.61	0.18	0.03	0.90-1.21	5	14
Fe, μmol/L	28.01	16.10	45.40	7.23	1.14	17.1-33.3[1]	9	1
ALP, U/L	295.54	27.50	457.40	102.04	16.13	99-464	2	1

*Lepherd *et al*. (2009); [1]Antunović *et al*. (2010c); ALP-alkaline phosphatase.

CONCLUSION

Determined biochemical parameters in the blood of Merinoladschaf lambs in organic farming can be used as a reliable criterion for assessing the supply of lambs with nutrients through the diets, and with redefining reference values for albumin, globulin, calcium and magnesium concentrations as well as CK activity, and their reference values.

ACKNOWLEDGEMENT

The research required for the present study were the part of the VIP-project financed by Ministry of Agriculture, Republic of Croatia.

REFERENCES

Antunović, Z., Novoselec, J., Klir, Ž. (2012): Ovčarstvo i kozarstvo u Republici Hrvatskoj – stanje i perspektive. Krmiva, 54(3): 99-109.

Antunović, Z., Novoselec, J., Šperanda, M., Senčić, Đ., Vegara, M., Steiner, Z. (2010a): Vanjština i biokemijski pokazatelji krvi Merinolandšaf ovce. Zbornik radova XXI Naučno-stručne konferencije poljoprivrede i prehrambene industrije. Neum, Bosna i Hercegovina, 29. 09 - 02. 11. 2010. pp. 22-23.

Antunović, Z., Novoselec, J., Šperanda, M., Domaćinović, M., Djidara, M. (2010b): Praćenje hranidbenog statusa janjadi iz ekološkog uzgoja. Krmiva, 52(1): 27-34.

Antunović, Z., Novoselec, J., Senčić, Đ., Šperanda, M., Steiner, Z., Samac, D. (2010c): Production traits and blood biochemical parameters in organic lamb production. Proceedings of the 45th Croatian & 5th International Symposium on Agriculture, Opatija, Croatia, pp. 1014-1018.

Antunović, Z., Šperanda, M., Senčić, Đ., Novoselec, J., Steiner, Z., Djidara, M. (2009): Influence of age some blood parameters of lambs in organic production. Macedonian Journal of Animal Science, 1(2): 11-15.

Antunović, Z., Senčić, Đ., Domaćinović, M., Šperanda, M., Steiner, Z. (2005): Changes in some blood parameters associated with the age of lambs. Medycyna Weterynaryjna, 61(7): 761-764.

Antunović, Z., Šperanda, M., Steiner, Z. (2004): The influence of age and the reproductive status to the blood indicators of the ewes. Archiv für Tierzucht-Archives of Animal Breeding, 47(3): 265-273.

Antunović, Z., Senčić, Đ., Šperanda, M., Liker, B. (2002): Influence of the season and the reproductive status of ewes on blood parameters. Small Ruminant Research, 45(1): 39-44.

Bickhardt, K., Duzfiak, D., Ganer, M., Henze, P. (1999): Dependence of hematological and chemical blood parameters on the age of healthy lambs: definition of reference values in sheep. Dtsch Tieratzl Woch, 106: 445-451.

Bojkovski, D., Simčić, M., Kompan, D. (2015): Supports for local breeds in the European region – an overview. Agriculture, 21(1): 7-10.

CAA-Croatian Agricultural Agency (2017): Annual report 2016: Sheep and goats. Križevci, pp. 88

Carlos, M. M. L., Leite, J. H. G. M., Chaves, D. F., Vale, A. M., Facanha, D. A. E., Melo, M. M., Soto-Blanco, B. (2015): Blood parameters in the Morada Nova sheep: influence of age, sex and body condition score. The Journal of Animal & Plant Sciences, 25(4): 950-955.

European Union (EU) 2007. Council Regulation (EC) No. 834/2007 on organic production and labeling of organic products with regard to organic production, labeling and control. Official Journal of the European Union. Available from: http://32007R0834.

Fernández, J. R., Ramos, E., De la Torre, G., Hermoso, R., Gil Extremera, F., Sanz Sampelayo, M. R. (2007): Blood metabolites as indicators of energy status in goats. In: Priolo A. (ed.), Biondi L. (ed.), Ben Salem H. (ed.), Morand-Fehr P. (ed.). *Advanced nutrition and feeding strategies to improve sheep and goat*. Zaragoza: CIHEAM, pp. 451-455. (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 74). 11. Seminar of the FAO-CIHEAM Sub-Network on Sheep and Goat Nutrition, 2005/09/08-10, Catania (Italy). http://om.ciheam.org/om/pdf/a74/00800412.pdf

Fayos, M., Couto, C. G., Iaybik, M. C., Wellman, M. L (2005): Serum protein electrophoresis in retired racing Greyhounds. Veterinary Clinical Pathology, 34: 397-400.

Hatfiled, P. G., Head, W. A., Fitzgerald, J. A., Hallford, D. M. (1999): Effects of level of energy intake and energy demand on growth hormone, insulin and metabolites in Tharghee and Suffolk ewes. Journal of Animal Science, 77: 2757-2765.

Herdt, T., Hoff, B. (2011): The use of blood analysis to evaluate trace mineral status in ruminant livestock. Veterinary Clinics of North America Food Animal Practice, 255-283.

Hrković, A., Hodžić, A., Hamamdžić, M., Vegara, M., Sarić, Z., Zahirovi, A., Juhas Pašić, E., Krnić, J. (2009): Karakteristike biokemijskog statusa bosansko-hercegovačke pramenke. Krmiva, 51(2): 117-126.

Kaneko, J. J., Harvey, J. W., Bruss, M. L. (2008): Clinical Biochemistry of Domestic Animals. 6th ed. Elsevier/Academic Press, Amsterdam, pp. 932.

Kiani, A. (2013): Temporal changes in plasma concentration of leptin, IGH-1, insulin and metabolites under extended fasting and re-feeding conditions in growing lambs. International Journal of Endocrinology & Metabolism, 11(1): 34-40.

Kincaid, R. L. (2000): Assessment of trace mineral status of ruminants: a review. Journal of Animal Science, 77: 1-10.

Kohn, R. A., Dinneen, M. M., Russek-Cohen, E. (2005): Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs and rats. Journal of Animal Science, 83: 879-889.

Lepherd, M. L., Canfield, P. J., Hunt, G. B., Bosward, K. L. (2009): Haematological, biochemical and selected acute phase protein reference intervals for weaned female Merino lambs. Australian Veterinary Journal, 87: 5-11

Roubies, N., Panousis, N., Fytianou, A., Katsoulos, P. D., Giadinis, N., Karatzias, H. (2006): Effects of age and reproductive stage on certain serum biochemical parameters of Chios sheep under Greek rearing conditions. J Vet Med A, 53: 277-281.

Russel, A. (1991): Body condition scoring of sheep. In: Sheep and goat practice. Boden E. (ed.). pp. 3. Bailliere Tindall, Philadelphia.

SAS 9.3 (2008): SAS Institute Inc., Cary, NC, USA.

Schweinzer, V., Iwersen, M., Drillich, M., Wittek, T., Tichy, A., Mueller, A., Krametter-Froetscher, R. (2017): Macromineral and trace element supply in sheep and goats in Austria. Veterinarni Medicina 62: 62-73.

Shek Vugrovečki, A., Vojta, A., Šimpraga, M. (2017): Establishing reference intervals of haematological and serum biochemical variables in Lika pramenka sheep. Vet Arhiv, 87: 487-499.

Tothova, C., Nagy, D. O., Kovac, G. (2016): Serum proteins and their diagnostic utility in veterinary medicine: a review. Veterinarni Medicina, 62(9): 475-496.

Tschuor, A. C., Riond, B., Braun, U., Lutz, H. (2008): Hämatologische und klinisch-chemische Referenzwerte für adulte Ziege und Scahfe. Schweiz Archive Tierheik, 287-295.

Van Saun, R. (2000): Blood profiles as indicators of nutritional status. Advance in Dairy Technology, 12: 401-410.

Van Saun, R. (2006): Metabolic profiles for evaluation of the transition period. Proceedings of the American Association of Bovine Practitioners, 39: 178-179.

VRIJEDNOSTI GONADOSOMATSKOG INDEKSA (GSI) I HISTOLOŠKA ANALIZA OVARIJA KALIFORNIJSKE PASTRMKE (*Oncorhynchus mykiss*) HRANJENE HRANOM DVA RAZLIČITA PROIZVOĐAČA*

GONADOSOMATIC INDEX VALUES AND HISTOLOGICAL ANALYSIS OF THE RAINBOW TROUT (*Oncorhynchus mykiss*) OVARY FED WITH FOOD FROM TWO DIFFERENT MANUFACTURERS

Selma Pilić[1], Anesa Jerković-Mujkić[1], Amela Katica[2], Nadžida Mlaćo[2]

Original scientific paper

Rezime

Bosna i Hercegovina je jedna od vodećih zemalja regiona u proizvodnji slatkovodnih riba, prvenstveno kalifornijske pastrmke (*Oncorhynchus mykiss* Walbaum, 1792). Faktori koji utiču na proizvodnju i reprodukciju kalifornijske pastrmke su brojni te jedan od njih je svakako vrsta i sastav hrane.

Komparativna studija gonadosomatskog indeksa (GSI) i ovarija kalifornijske pastrmke hranjene hranom različitih proizvođača provedena je na Magazin Maprim i Eko-projekt ribogojilištu u kojima je bio zastupljen betonski način uzgoja. Na Magazin Maprim ribogojilištu bila je zastupljena peletirana riblja hrana proizvođača Coppens (Holandija), dok je na Eko-projekt ribogojilištu korištena Skretting (Italija). Praćene su dvije grupe od po 30 jedinki iste uzrasne dobi. Fizičko-hemijski parametri vode bili su unutar prihvatljivih granica za uzgoj ove vrste.

Rezultati su pokazali da GSI (16,44±0,65) ženki hranjenih hranom Coppens nije pokazao statistički signifikantnu razliku od GSI (15,98±1,41) ženki hranjenih hranom Skretting. Srednja težina ovarija (270±0,04) ženki hranjenih hranom Coppens pokazala je statistički značaj u poređenju sa težinom ovarija ženki (177±0,02) hranjenih hranom Skretting. Histologija ovarija kalifornijske pastrmke podvrgnute eksperimentalnim hranama pokazala je normalan razvoj i distribuciju jajnih i intersticijskih stanica.

Na temelju ovih nalaza, hrana proizvođača Skretting i proizvođača Coppens su prilično slične po svom učinku na GSI indeks i histologiju ovarija kalifornijske

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Faculty of Science, University of Sarajevo, Department of Biology, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina
[2] Veterinary Faculty, University of Sarajevo, Department of Histology and Embriology, Zmaja od Bosne 90, 71000 Sarajevo, Bosnia and Herzegovina

pastrmke te s obzirom na jednaku dostupnost hrane oba proizvođača preporučuje se uzgajivačima ribe hrana koja je financijski ekonomičnija.

Ključne riječi: *Oncorhynchus mykiss, GSI, težina ovarija, peletirana riblja hrana proizvođača Coppens i Skretting*

Summary

Bosnia and Herzegovina is one of the leading countries in the region in the production of freshwater fish, primarily rainbow trout (*Oncorhynchus mykiss*). Factors affecting the production and reproduction of rainbow trout are numerous, and the type and composition of the food being most certainly one of them.

Comparative study of gonadosomatic index (GSI) and ovary of rainbow trout, fed with food from two different producers was conducted at Magazine Maprim and Eco-project fish farms, where concrete tanks for breeding are used. The Coppens food was used in Magazine Maprim fish farm, while in Eco-project fish farm, the Skretting food was used. Two groups of 30 individuals of the same age were examined. Physicochemical parameters of water were within acceptable values for this species.

The results showed that GSI (16,44±0,65) values for females fed with Coppens food did not differ statistically from GSI (15,98±1,41) values for females fed with Skretting. When mean ovary weight was analyzed, statistically significant difference was observed between females fed with Coppens food (270±0,04) and females fed with Skretting food (177±0,02). Histology of ovaries of the rainbow trout fed with the two experimental diets showed normal development and distribution of oocytes and interstitial cells.

Based on our results, food from Skretting and Coppens manufacturers are quite similar regarding their effect on GSI index and ovary histology of the rainbow trout. Given equal availability of these two goods, we recommend to fish farmers to use the one which is more economical.

Key words: *Oncorhynchus mykiss, GSI, Ovary weight, Coppens and Skretting fish food*

UVOD

Posljednjih nekoliko decenija akvakultura u svijetu se izdvaja kao najbrže rastući sektor proizvodnje hrane. Najveći dio te proizvodnje odvija se u slatkovodnim ribnjacima (Ottolenghi *et al.*, 2004). Bosna i Hercegovina je jedna od vodećih zemalja regiona u proizvodnji slatkovodnih riba, prvenstveno kalifornijske pastrmke (*Oncorhynchus mykiss* Walbaum, 1792). Trend povećanja proizvodnje prema raspoloživim podacima Agencije za statistiku Bosne i Hercegovine (2012) nastavlja se iz godine u godinu.

Kalifornijska pastrmka uzgaja se u betonskim ili kaveznim sistemima i po hektaru bazena može postići prinos od 100.000 do 500.000 kg (Hamzić, 1993). Fenotipska plastičnost njenih bioloških svojstava je glavni razlog za prevladavanje ove vrste u intenzivnom uzgoju. Proizvodni rezultati su u visokom stepenu zavisnosti od mikroklimatskih uslova uzgojne sredine (temperature, koncentracije rastvorenog kiseonika te pH vrijednosti vode), kvaliteta hrane, nivoa ishrane (Akbary *et al*., 2010; McMillan *et al*., 2012) i genetičkog potencijala rasta kalifornijske pastrmke (Pante *et al.*, 2002). U vještačkom uzgoju kalifornijske pastrmke, ishrana je, bez sumnje najteži i najsloženiji poduhvat. Složenost problema ishrane proizilazi, ne toliko iz odabira vrste i količine hrane, nego više iz činjenice da je ona uslovljena nizom raznih faktora, kao što su: karakter i lokacija objekta, protok i temperatura vode, sadržaj rastvorenog kiseonika u vodi, veličina i stepen razvitka riba, gustina naseljenosti, porijeklo i zdravstveni status ribe. Visoka produktivnost zasnovana je na balansiranoj i pravilnoj ishrani sa svim komponentama koje potpomažu intenzivan rast i sprječavaju pojavu oboljenja (Willoughby, 1999). Neznatne promjene uslova držanja i ishrane remete iskorištavanje biološkog potencijala riba, a preko određenih granica ugrožavaju fiziološke funkcije, odnosno dovode do poremećaja zdravstvenog stanja organizma (Jeremić, 2003). Uzgoj kalifornijske pastrmke je veoma rentabilan i značajan dio akvakulture, koji ima za cilj snabdijevanje tržišta visokokvalitetnim proteinima animalnoga porijekla (Vranić *et al*., 2011). Hranjiva vrijednost mesa riba uslovljena je količinom proteina, masti, minerala i vitamina u njemu i zavisi od starosti ribe, načina uzgoja, sastava hrane i godišnjeg doba izlova.

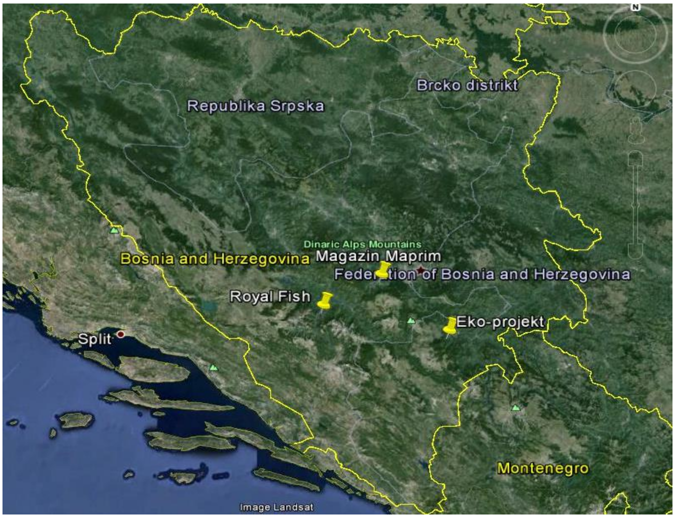
U literaturi se može naći nekoliko studija koje su se bavile ispitivanjem uticaja različitih vrsta hrane na proizvodne i reproduktivne performanse ribljih vrsta (Ahmadi *et al*., 2006; Ekanem *et al.,* 2012; Ekanem *et al.,* 2013; Eyo *et al*. 2014).

Cilj ove studije bio je komparirati gonadosomatski indeks (GSI) i histološku deskripciju ovarija kalifornijske pastrmke hranjene peletiranom ribljom hranom dva proizvođača Coppens i Skretting.

MATERIJAL I METODE

Istraživane jedinke. Studija je obuhvatila dvije grupe od po 30 ženki kalifornijske pastrmke (*Oncorhynchus mykiss* Walbaum, 1792). Jedinke su uzorkovane sa dva ribogojilišta: Magazin Maprim i Eko-projekt. Za uzorkovanje riba koristio se ribarski sak. Starost uzorkovanih jedinki kalifornijske pastrmke određivana je na osnovu krljušti.

Istraživana ribogojilišta. Istraživanja su provedena na Magazin Maprim i Eko-projekt ribogojilištu sa klasičnim intenzivnim uzgojom ribe u betonskim bazenima. Ribogojilišta se nalaze na tokovima koji pripadaju različitim slivovima, shodno tome imaju i različite mikroklimatske uvjete okoliša. Ribogojilište Magazin Maprim smješteno je na toku Ramičkog potoka koji se ulijeva u rijeku Bosnu. Eko-projekt ribogojilište smješteno je na toku rijeke Krupice koja se zajedno sa Govzom ulijeva u Bistricu, a zatim u Drinu (Slika 1). Na ribogojilištima su provedena mjerenja osnovnih fizičko-hemijskih parametara kvaliteta vode (temperature, koncentracije rastvorenog kiseonika te pH vrijednosti) pomoću aparata marke Multi 340i/SET (Germany).



Slika 1. Fizičko-geografski položaj ribogojilišta na kojima su provedena istraživanja

Na Magazin Maprim ribogojilištu bila je zastupljena peletirana riblja hrana proizvođača Coppens (Holandija), dok je na Eko-projekt ribogojilištu korištena hrana tvrtke Skretting (Italija). Zastupljenost nutrijenata u peletiranim ribljim hranama korištenim za ishranu predkonzumnih i konzumnih kategorija riba na istraživanim ribogojilištima predstavljena je u tabeli 1.

Tabela 1. Komparativni prikaz sastava peletirane riblje hrane

Proizvođač	Peletirana riblja hrana	Sirovi proteini (%)	Sirova mast (%)	Sirova vlakna (%)	Pepeo (%)	Fosfor (%)	Vitamin A IU/kg	Vitamin D3 IU/kg
Skreting (Italija)	Skreting optiline HE 2P	42	26	3	4,0	0,6	5000	1 000
Cooppens (Holandija)	Ultra troco	43	28	1	8,3	0,9	15 000	3 000

Određivanje gonadosomatskog indeksa. Za određivanje tjelesne mase i mase ovarija korištena je digitalna analitička vaga (Tehtnica ET 1111) sa mjernom tačnošću na dvije decimale. Mjerenja su vršena u laboratoriji za citologiju Prirodno-matematičkog fakulteta Sarajevo. Razvoj gonada određen je upotrebom gonadosomatskog indeksa (GSI), koji je izračunat uz pomoć formule (Bolger & Connolly, 1989):

GSI = težina gonada (g) / ukupna težina ribe (g) * 100

Izrada i analiza mikroskopskih preparata. Nakon disekcije, te odvajanja ovarija od okolnog tkiva gonade su fiksirane u 10% formaldehidu. Mikroskopski preparati su izrađivani i analizirani u laboratoriji za Histologiju i embriologiju Veterinarskog fakulteta u Sarajevu. Postupak obrade uzoraka od fiksacije do kalupljenja u parafin proveden je na rotacijskom procesoru tkiva (MICROM model STP 120). Nakon završenog procesa kalupljenja, uzorci su rezani digitalnim mikrotomom (LEICA RM 2145) u više serijskih rezova debljine od 0,5 do 1,5 mikrometar i obojeni standardnom metodom hematoksilin-eozina (Bancroft & Cook, 1994). Pregled histoloških preparata vršili smo svjetlosnim mikroskopom opremljenim kamerom marke MOTIC TYPE 102M, pod uvećanjem od 100, 200 i 400x. Provedene analize histoloških struktura su izvršene primjenom posebnog programa Motic Images Plus 2.0 ML.

Statistička analiza. Na osnovu dobivenih podataka iz ribogojilišta urađene su statističke analize pomoću softvera Statistika 8.0 (© Copyright StatSoft, Inc. 1984-2007). Provedena morfometrijska mjerenja urađena su u većem broju ponavljanja, a rezultati prikazani kao aritmetičke sredine više mjerenja sa standardnom devijacijom (Microsoft Excel 2007). Jednosmjerna ANOVA (*One way* ANOVA) primijenjena je za komparaciju posmatranih varijabli, nakon čega je slijedio Post-hoc Newman-Keuls test. Za navedene analize primijenjen je statistički nivo značajnosti od $p<0,05$.

REZULTATI ISTRAŽIVANJA

Izmjerene vrijednosti fizičko-hemijskih parametara vode na istraživanim ribogojilištima bili su unutar prihvatljivih granica za uzgoj kalifornijske pastrmke. Na osnovu tvorničke deklaracije sastava peletirane riblje hrane korištene na istraživanim ribogojilištima nisu uočene značajnije razlike u sadržaju sastavnih komponenti hrane proizvođača Skretting i proizvođača Coppens (Tabela 1).

Rezultati su pokazali da GSI (16,44±0,65) ženki hranjenih hranom proizvođača Coppens nije pokazao statistički signifikantnu razliku od GSI (15,98±1,41) ženki hranjenih hranom proizvođača Skretting. Srednja težina ovarija (270±0,04) ženki hranjenih hranom Coppens pokazala je statistički značaj u poređenju sa težinom ovarija ženki (177±0,02) hranjenih hranom Skretting (Slika 2,3).

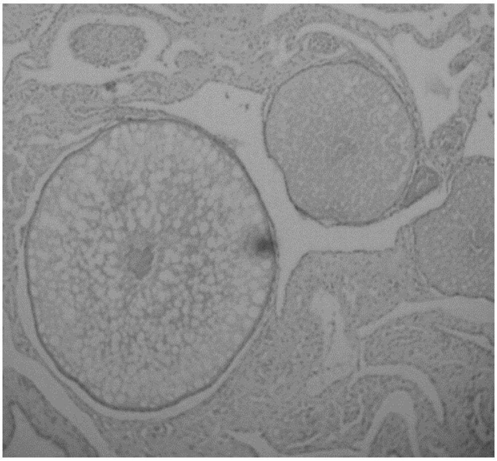
Histološka istraživanja ovarija kalifornijske pastrmke podvrgnute eksperimentalnim hranama pokazala su normalan razvoj i distribuciju jajnih i intersticijskih stanica. Histološkom deskripcijom ovarija kalifornijske pastrmke sa odabranih ribogojilišta uočene su i blage razlike u intenzitetu oogeneze, ali oocite su se mogle zamijetiti u svim razvojnim fazama oogneze (Slika 4a, 4b, 4c, 4d).

Slika 2. Ovarij *O. mikiss* hranjenih Coppens hranom

Figure 2. Ovary of O. mykiss fed with Coppens food

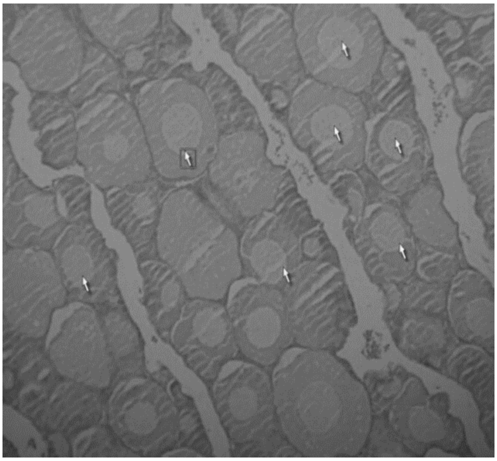
Slika 3. Ovarij *O. mikiss* hranjenih Skretting hranom

Figure 3. Ovary of O. mykiss fed with Skreting food



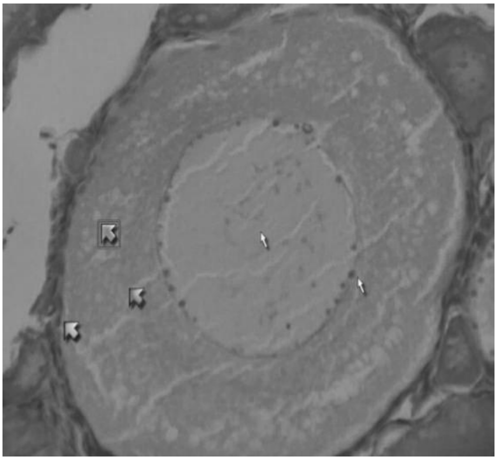
4a. Generalna slika ovarija u vrijeme mrijesta: vitelogene oocite (HE X 200)

4a. General figure of ovary during spawn: vitelogen oocytes (HE X 200)

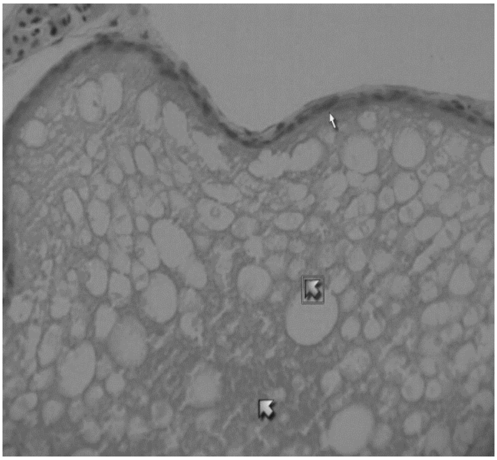


4b. Perinuklearna faza: bijele strelice označavaju primarne oocite (HE X 100)

4b. Perinuclear phase: white arrows shows primary oocytes (HE X 100)



4c. Primarna oocita: jedro i jedarca-bijele strelice; zona granuloza-zelena strelica; žumančane granule-crvena strelica; vakuole-žuta strelica (HE X 400)
4c. Primary oocyte: nucleus and nucleolus-white arrows; granular area-green arrow; egg yolk granules-red arrow; vacuoles-yellow arrow (HEX400)



4d. Sekundarna žumančana oocita: zona radijata-bijela strelica; žumančane granule-zelena strelica; vakuole-crvena strelica (HE X 400)
4d. Secondary oocyte: zona radiata-white arrow; egg yolk granules-green arrow; vacuole-red arrow (HE X 400)

DISKUSIJA

Hranidbene potrebe za nutrijentima (proteini, lipidi, ugljikohidrati, minerali i vitamini) kao i optimalne granične vrijednosti okolišnih faktora specifične su za pojedine vrste, pa čak i za razvojne stadije iste vrste (Katavić, 2009). Detaljno poznavanje problema ishrane znači voditi računa ne samo o dužinskom i masenom prirastu te ekonomičnosti uzgoja salmonidnih vrsta riba, nego i o svim njihovim fiziološkim i patološkim stanjima, nastojeći pronaći moguće greške u uzgoju. Naime, poznavanje fiziologije probave i ishrane tj. mogućnosti iskorištavanja i potrebe za pojedinim hranjivim materijama, pri različitim ekološkim uslovima, omogućavaju da se i bez prethodnih ogleda iz dostupnih hranjiva sastavi kvalitetna hrana. Ova saznanja su naročito važna i vrijedna pri određivanju sastava granulirane i peletirane hrane. Pravilan izbor hrane i količina dnevnog obroka od podjednake su važnosti. Oni ne samo da određuju visinu prirasta i cijenu proizvedene ribe, nego i značajno utiču na kvalitet vode (Dabrowski *et al.*, 2000; Ćirić, 2013). Zbog toga se mora voditi računa o svim aspektima prehrane uz osiguranje optimalnih uvjeta okoliša (Ahmadi *et al.*, 2006; Mireşan *et al.*, 2010). Pri ocjenjivanju kvaliteta hrane za ribe, uglavnom je glavni kriterij tempo rasta (Ekanem *et al.*, 2012a; Ekanem *et al.*, 2012b). Različite vrste hrane, pri istim fizičko-hemijskim parametrima vode, dovode do različitog masenog prirasta riba kao rezultat različitog udjela sastavnih komponenti (Ekanem *et al.*, 2013). Iz tog razloga smo u našem istraživanju upravo odabrali peletiranu riblju hranu dva različita proizvođača koji su najčešće zastupljeni na ribogojilištima u Bosni i Hercegovini, u cilju pružanja korisnih informacija i poboljšanja određenih uslova života u cilju produktivnijeg mrijesta i uzgoja. U provedenoj studiji fizičko-hemijski parametri vode bili su unutar prihvatljivih granica za uzgoj ove vrste. Utvrđene vrijednosti gonadosomatskog indeksa (GSI) i histološka analiza ovarija kalifornijske pastrmke hranjene hranom dva različita proizvođača nisu pokazali značajnije razlike. Dobijeni rezultati su u skladu sa podacima studije provedene od strane Eyo *et al.* (2012). Mnogobrojne studije su proučavale uticaj hrane ili uticaj različitog sastava hrane na prirast ribe, plodnost i gametogenezu kao i na kvalitet gameta (Brooks *et al.*, 1997; Blom *et al.*, 2001; Izquierdo *et al.*, 2001; Ekanem *et al.*, 2012b). U nekoliko studija došlo se do zaključka da ribe hranjene hranom životinjskog porijekla imaju brži težinski i dužinski prirast, u odnosu na ribe hranjene hranom biljnog porijekla (Ekanem *et al.*, 2012a). Takođe, konstatovane su razlike u plodnosti, rastu i razvoju gonada te gonadosomatskog indeksa u korist hrane sa sastojcima životinjskog porijekla. Rezultati dosadašnjih studija govore u prilog činjenici da visoku produktivnost i bolje reproduktivne performanse uzgoja salmonidnih vrsta riba svakako osigurava hrana visoke kvalitete.

ZAKLJUČAK

Na osnovu provedenog istraživanja možemo konstatovati da su peletirane riblje hrane proizvođača Skretting i Coppens vrlo slične po svom učinku na GSI indeks. Histološka deskripcija ovarija pokazala je normalnu distribuciju stanica i njihov razvoj. S obzirom na jednaku dostupnost hrane oba proizvođača, preporučuje se uzgajivačima ribe hrana koja je financijski ekonomičnija.

LITERATURA

Agencija za statistiku Bosne i Hercegovine (2012). Aquaculture. *Published and printed by the Agency for Statistics of the Bosnia and Herzegovina*. First release.

Ahmadi, M. R., Bazyar, A. A., Safi, S., Ytrestøyl, T., Bjerkeng, B. (2006). Effects of dietary astaxanthin supplementation on reproductive characteristics of rainbow trout (*Oncorhynchus mykiss*). *Journal of Applied Ichthyology*, 22(5): 388-394.

Akbary, P., Hosseini, S. A., Imanpoor, M., Sudagar, M., Makhdomi, N. M. (2010). Comparison between live food and artificial diet on survival rate, growth and body chemical composition of *Oncorhynchus mykiss* larvae. *Iranian Journal of Fisheries Sciences*, 9(1): 19-32.

Bancroft, J. D., Cook, H. C. (1994). Manual of histopathological techniques and their diagnostic application. Churchill Livingstone, London, pp. 305.

Blom, J. H., Lee, K. J., Rinchard, J., Dabrowski, K., Ottobre, J. (2001). Reproductive efficiency and maternal-offspring transfer of gossypol in rainbow trout

(*Oncorhynchus mykiss*) fed diets containing cottonseed meal. *J. Anim. Sci.,* 79(6): 1533-1539.

Bolger, J., Connolly, P. L. (1989): The selection of suitable indices for the measurement and analysis of fish condition. *Journal of Fish Biology,* 34, 171-182.

Brooks, S., Tyler, C. R., Sumpter, J. P. (1997). Egg quality in fish: what makes a good egg? *Revi. Fish Biol. Fish.,* 7: 387-416.

Ćirić, M. D. (2013). Uticaj različitih tipova dodatne hrane u poluintenzivnoj proizvodnji mlađi šarana (*Cyprinus carpio*) na strukturu i dinamiku ribnjačkog ekosistema. Doktorska disertacija. Poljoprivredni fakultet Univerziteta u Beogradu.

Dabrowski, K., Rinchard, J., Lee, K-J., Blom, J. H., Ciereszko, A., Ottobre, J. (2000). Effects of Diets Containing Gossypol on Reproductive Capacity of Rainbow Trout (*Oncorhynchus mykiss*). *Biology of reproduction,* 62: 227-234.

Ekanem, A. P., Eteng, S. U., Nwosu, F. M., Eyo, V. O. (2012a). Comparative Study of the Growth and Gonad Development of *Clarias gariepinus* (Burchell 1822) Fed Diets with Plant and Animal-based Ingredients in Concrete Tanks. *Journal of Agricultural Science and Technology,* A2: 1203-1210.

Ekanem, A. P., Eyo, V. O., Obiekezie., A. I., Enin Udeme, I., Udo, P. J. (2012b). A Comparative Study of the Growth Performance and Food Utilisation of the African Catfsh (*Clarias gariepinus*) Fed Unical Aqua Feed and Coppens Commercial Feed. *Journal of Marine Biology & Oceanography,* 1(2): 1-6.

Ekanem, A. P., Eyo, V. O., James, P., Udoh, U., Nsisong, E., (2013). Effects of Unical Feed on Fecundity and Gonad Development of *Clarias gariepinus*; A Comparative Study with Coppens Commercial Feed in Earthen Pond. *International Journal of Science and Research (IJSR)*, 2(10): 8-14.

Eyo, V. O., Ekanem, A. P., Obiekezie, A. I. (2012). A comparative study of egg and sperm quality of *Clarias gariepinus* fed Unical Aqua Feed and Coppens Commercial Feed. *IJBPAS*, 1(11): 1598-1607.

Eyo, V. O., Ekanem, A. P., Ufonima Udo, J. (2014). A comparative study of the gonado-somatic index (GSI) and gonad gross morphology of african catfish (*Clarias gariepinus*) fed Unical Aqua feed and Coppens Commercial feed. *Croatian Journal of Fisheries,* 72: 63-69.

Hamzić, A. (2003). Akvakultura u Bosni i Hercegovini. Corons d.o.o. Sarajevo.

Izquierdo, M. S., Ndez-Palacios, H., Tacon, A. G. J. (2001). Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture,* 197: 25-42.

Jeremić, S. (2003). Uticaj ekoloških činioca sredine kao stres faktori na zdravlje riba. *Savremena poljoprivreda,* 52(3–4): 465-470.

Katavić, I. (2009). Okolišni aspekti akvakulture s posebnim osvrtom na organski otpad i prihvatni kapacitet uzgajališta. Uzgoj slatkovodne ribe, stanje i perspektive zbornik radova, Hrvatska gospodarska komora, Sektor za poljoprivredu, prehrambenu industriju i šumarstvo. Udruženje ribarstva i prerade ribe, Zagreb.

McMillan, J. R., Dunham, J. B., Reeves, G. H., Mills, J. S., Jordan, C. E. (2012). Individual condition and stream temperature influence early maturation of rainbow and steelhead trout, *Oncorhynchus mykiss*. *Environmental Biology of Fish*, 93: 343-355.

Mireșan, V., Cocan, D., Chirilean, I., Constantinescu, R., Răducu, C., Festilă, I., Sărmas, I. (2010). Morpho-Histological Peculiarities of Oro-Pharyngeal Cavity of Rainbow Trout (*Oncorhynchus mykiss*, Wallbaum 1792). *Animal Science and Biotechnologies*, 67(1-2): 469.

Ottolenghi, F., Silvestri, C., Giordano, P., Lovatelli, A., New, M. B. (2004). Capture-based aquaculture: The Fattening of eels, groupers, tunas and yellowtails, Food and Agriculture Organization of the United Nations, Rim.

Pante, M. J. R., Gjerde, B., McMillan, I., Misztal, I. (2002). Estimation of additive and dominance genetic variance for body weight at harvest in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture Journal*, 204: 383-392.

Vranić, D., Đinović-Stojanović, J., Spirić A. (2011). Kalifornijska pastrmka (*Oncorhynchus mykiss*) iz akvakulture-kvalitet mesa i značaj u ishrani. *Tehnologija mesa*, 52(1): 122-133.

Willoughby, S. (1999). Manual of Salmonids Farming. Fishing new books. Blackwell Science.

Radovi Poljoprivredno-prehrambenog fakulteta Univerziteta u Sarajevu, God. LXII, broj 67/2
Works of the Faculty of Agriculture and Food Sciencies, University of Sarajevo, Vol. LXII, No. 67/2

PROMJENE HEMATOLOŠKIH PARAMETARA *Oncorhynchus mykiss* (Walbaum, 1792) PRIJE I NAKON ZIMSKOG PERIODA

HEMATOLOGICAL CHANGES *Oncorhynchus mykiss* (Walbaum, 1792) BEFORE AND AFTER WINTER PERIOD

Maja Mitrašinović-Brulić[1], Maira Burić[1], Rifat Škrijelj[1], Senad Šljuka[1], Jasmin Mušanović[2]

Original scientific paper

Rezime

Opće je poznato da hipotermija inducira kod riba hronično stanje stresa, koje se može procijeniti prateći promjene hematoloških parametara, koji variraju ovisno o intenzitetu stresa. Riba, kao poikilotermni organizam s niskom razinom homeostaze, podvrgnuta je različitim nepovoljnim promjenama u procesu uzgoja. Ove su promjene uglavnom tehnološke prirode: nepravilna prehrana, česte manipulacije, veliki broj riba u ribnjacima, nedovoljan protok vode, nepoželjni fizički i hemijski indeksi, te primjena preventivnih i ljekovitih biljaka. Svi gore navedeni faktori uzrokuju stresne reakcije. Fiziološki odgovor riba na stres očituje se u slijedećem: oslobađanje kateholamina (adrenalina i noradrenalina) i kortizola, što dovodi do promjena u metabolizmu, kardiovaskularnom i imunološkom sistemu, rastu i ponašanju. Stres dovodi do mnogobrojnih biohemijskih reakcija koje počinju u hipotalamusu i području hipofize mozga. Oni stimuliraju nadbubrežnu žlijezdu i povećavaju oslobađanje adrenalina koji stimulira glikogenolizu u jetri i mišićima, što uzrokuje hiperglikemiju i povećanu koncentraciju mliječne kiseline u mišićima. Krvna slika riba reflektira fiziološko stanje organizma. U ovom istraživanju korišteni su uzorci 90 jedinki kalifornijske pastrmke *Oncorhynchus mykiss* iz ribnjaka "Eko-Fish" (6 km jugoistočno od Jablanice, u selu Glogošnica) u razdoblju od septembra 2013. do marta 2014. godine. Ispitivani su sljedeći hematološki parametri: koncentracija hemoglobina, vrijednost hematokrita, broj eritrocita, prosječni volumen eritrocita (MCV), prosječna vrijednost količine hemoglobina po eritrocitu (MCH), prosječna vrijednost količine hemoglobina po litri eritrocita (MCHC) kao i broj leukocita. Statistička analiza provedena je korištenjem IBM SPSS *Statistics 20*. Stanje hipotermije utiče na vrijednosti hematoloških parametara kalifornijske pastrmke, te dovodi do povećanja vrijednosti hematokrita, MCV i broja leukocita, dok se smanjuje broj eritrocita, koncentracija hemoglobina, MCH i MCHC. Analiza navedenih parametara po spolu, pokazuje znatno veće vrijednosti hemoglobina i hematokrita kod mužjaka kalifornijske pastrmke.

[1] Odjek za biologiju, Prirodno-matematički fakultet, Univerzitet u Sarajevu
[2] Medicinski fakultet, Univerzitet u Sarajevu

Ključne riječi: *kalifornijska pastrmka, hematološki parametri, stres, hipotermija*

Summary

It is already know that, hypothermia induce in fish a chronic winter state of stress, which may be estimate through hematological insufficiencies and which is demonstrated commensurable in the stress intensity. Fish, as poikilothermic organism with low level of homeostasis, are subjected to various adverse changes in the process of breeding. These changes are mostly of a technological nature: mistakes in diet, frequent manipulation, large number of fish in ponds, insufficient water flow, undesirable physical and chemical indexes and application of preventive and medicinal plants. All aforementioned factors cause stressful reactions. Their physiological response to stress is manifested in the following: release of catecholamine (adrenaline and noradrenaline) and cortisol; which leads to changes in metabolism, cardiovascular and immune system, growth and behavior. Stress leads to various biochemical reactions that start in hypothalamus and pituitary regions of brain. They stimulate the adrenal gland and increase the release of adrenaline. Adrenaline stimulates glycogenolysis in liver and muscles, which causes hyperglycemia and increased concentration of lactic acid in muscles. Fish blood cell count reflects the physiological state of the organism. This study include 90 individuals of rainbow trout specimens utilized in this research are from fish farm „Eko-Fish“ (which is 6 km south east of Jablanica, in the village of Glogošnica) and presents the hematological results of comparative determinations during the period from September 2013 to March 2014. The following hematological parameters were examined: haemoglobin concentration, haematocrit value, number of erythrocytes, average volume of erythrocytes (MCV), average value of haemoglobin quantity per erythrocyte (MCH), average value of haemoglobin quantity per liter of erythrocyte (MCHC) as well as the number of leukocytes. Statistical analysis was performed using the *IBM SPSS Statistics 20.* Hypothermia affects the blood stream of rainbow trout, and leads to an increased haematocrit value, MCV and the number of leukocytes, while it reduces the number of erythrocytes, haemoglobin concentration, MCH and MCHC. Sex-based analysis presents considerably higher levels of haemoglobin and haematocrit value in male rainbow trout.

Key words: *rainbow trout, hematological parameters, stress, hypothermia*

UVOD

Kalifornijska ili dužičasta pastrmka (*Oncorhynchus mykiss,* Walbaum, 1792) je porijeklom iz Kalifornije, a u Bosnu i Hercegovinu je prenesena 1902-1904. godine kada je dobavljena ikra ove vrste i nasađena u gojilište pastrmke na Vrelu Bosne. Ista mlađ je 1904. godine nasađena u Boračko jezero, a time i u sliv Neretve. Cijelo tijelo joj je prekriveno tamnim pjegama, raste od 35 do 70 cm dužine, a teži od 0,5 do 6 kg

(Škrijelj, 2002). Područje Bosne i Hercegovine, karakteriše dominantno komercijalno gajenje dužičaste pastrmke zbog izraženih karakteristika rasta i povoljnih uslova za njeno gajenje koji se prvenstveno ogledaju kroz kvalitet i količinu raspoložive vode. Hipotermija izaziva stresnu reakciju i predstavlja stanje pri kojem tjelesna temperatura slučajno ili namjerno opada za više od 2 °C ispod normalne (optimalne, zdrave) razine. Optimalna temperatura vode za kalifornijsku pastrmku se kreće u rasponu od 12 do 20 °C (Škrijelj, 2002). Bogut i sar., (2006) navode da hipotermija može dovesti do hipoglikemije, povišenog krvnog pritiska i smanjenja srčane frekvencije. Pored navedenih reakcija pod dejstvom hipotermije, pomenuti autori navode i često pojačano izlučivanje urina, smanjenje funkcije leukocita i u konačnici može dovesti do groznice i smrti. Tseng i sar., (2014) indiciraju da hipotermija smanjuje metaboličke stope kod svih životinja. Stres dovodi do kaskadnog slijeda biohemijskih reakcija koje započinju u dijelovima mozga označenim kao hipotalamus i hipofiza, a čiji je krajnji rezultat stimulacija nadbubrežne žlijezde i pojačano lučenje adrenalina (Selye, 1985). Adrenalin stimulira glikogenolizu u jetri i mišićima, i dovodi do hiperglikemije i povećanja koncentracije mliječne kiseline u mišićima (Miholjčić i sar., 1988). Gotovo svaka vrsta stresa uzrokuje trenutno i snažno pojačanje lučenja adrenokortikotropnog hormona (ACTH) iz adenohipofize, nakon čega se za nekoliko minuta znatno poveća lučenje kortizola iz kore nadbubrežnih žlijezda. Kortizol potiče glukoneogenezu, te povisuje koncentraciju glukoze u krvi. Izlučivanje kortizola u stresnim situacijama je korisno jer kortizol uzrokuje brzu mobilizaciju aminokiselina i masti iz depoa u stanicama čineći ih tako odmah dostupnim za oslobađanje energije i za sintezu glukoze (Cherniack i sar., 1991; Guyton, 2003). Međutim, previsoka koncentracija kortizola u krvi smanjuje odbrambene reakcije organizma. Kod riba se tjelesna tekućina smrzava na –0,8 °C (Škrijelj i Đug, 2009). Da bi bolje izdržale niske temperature, polarne vrste riba u krvi i tkivima imaju antifriz peptide (AFP) ili glikopeptide (AFGP) koji omogućavaju tjelesnoj temperaturi da se spusti do < - 0,8 °C bez smrzavanja (Dekić i sar., 2013). Ako su sposobne proizvoditi ove specijalne molekule, mnoge od ovih riba mogu tolerisati temperaturu do – 1,9 °C (Kock, 1992; Pörtner, 2002). Kestin i sar., (1991) su izvijestili da kalifornijska pastrmka ugine nakon 196 min na temperaturi od 2 °C.

Hematološki parametri su dobri pokazatelji odgovora ribe na stres, te se mogu koristiti i u evaluaciji njihovog zdravstvenog stanja pri nepovoljnim uslovima (Iversen i sar., 1998). Stres izaziva povećanje vrijednosti hematokrita, broja eritrocita i MCV, te smanjenje vrijednosti hematološkog indeksa MCHC. Visoka vrijednost broja eritrocita obezbjeđuje bolji dotok kisika do tkiva, brži metabolizam i potencijalno veće fizičke sposobnosti. Porast broja eritrocita po jedinici volumena krvi može biti posljedica deficita kisika tokom transporta i/ili aklimatizacije.

Kapacitet vezivanja kisika u krvi prvenstveno ovisi o koncentraciji i svojstvima hemoglobina sadržanog u eritrocitima (Ivanova, 1983). Davis i sar., (2008) smatraju da dolazi do velikih promjena u leukocitnom profilu riba usljed povećanog stresa. Cilj ovog rada je bio utvrditi hematološki status kalifornijske pastrmke

(*Oncorhynchus mykiss*, Walbaum 1792) i granice njegovog fiziološkog variranja prije i nakon zimskog perioda, te istražiti postojanje spolne specifičnosti vrijednosti navedenih parametara.

MATERIJAL I METODE

Jedinke vrste kalifornijske pastrmke korištene za ova istraživanja donešene su iz ribogojilišta „Eko-Fish", smješteno 6 kilometara sjeveroistočno od Jablanice, u selu Glogošnica, na rijeci Draganjki, u periodu od septembra 2013. do marta 2014. godine. Navedeno ribogojilište je polusistemski ribnjak specijalizovan za gajenje riba od mlađi do konzumne veličine. Sadrži ukupno 10 uzgajališnih bazena čija dužina iznosi 20 m, a širina 4 m i dva mrijestilišta. Mrijest se odvija u jesenskim i zimskim mjesecima, tačnije od polovine novembra do kraja decembra. U ovom ribogojilištu temperatura vode iznosi 13 °C, koncentracija kisika 11 mg/l, a pH vrijednost 7,4. Izlov ribe korištene u ovom radu vršen je primjenom standarnih lovnih metoda u ribnjaku, tačnije primjenom rogača. Ovakav način izlova ribe ne dovodi do uginuća, a stres kod ribe izazvan ovom lovnom metodom je blag i kratkotrajan. Nakon ulova jedinke su stavljene u namjensku posudu (bure) s vodom uzetom na licu mjesta koja je permanentno obogaćivana kisikom aerisanjem, uz korištenje odgovarajućeg aeratora. Ribe su transportovane u laboratorij Prirodno-matematičkog fakulteta u Sarajevu, gdje su vršene dalje eksperimentalne metode. Za potrebe ovog istraživanja uzeti su uzorci krvi (n=90) jedinki kalifornijske pastrmke (40 za kontrolu (septembar) i 50 jedinki za eksperiment (mart), koje su prebačene u akvarij sa dva aeratora. Temperatura vode je iznosila: za kontrolnu grupu 13°C (ista temperatura kao i u ribogojilištu), dok je temperatura vode u zimskom periodu iznosila 3 °C. Uzimanje krvi za hematološke analize vršilo se punktiranjem srca oštrom i širokom sterilnom iglom (1.0 do 1.2 mm). Prilikom uzimanja krvi vršena je dezinfekcija mjesta uboda, a prilikom punktiranja su bila primijenjena sva pravila sterilnog rada. Nativna krv bez dodatka antikoagulativnog sredstva koristila se za dalju analizu. Od hematoloških parametara praćeni su: koncentracija hemoglobina (Hb), vrijednost hematokrita (Hct), broj eritrocita (RBC), prosječna zapremina eritrocita (MCV), srednja vrijednost količine hemoglobina po eritrocitu (MCH), srednja vrijednost količine hemoglobina po litri eritrocita (MCHC) i broj leukocita (WBC). Broj eritrocita i leukocita određen je standardnom metodom brojanja u hemocitometru po Neubaueru. Ovaj postupak zahtijeva korišćenje melanžera (prilagođene pipete za brojanje eritrocita i leukocita) i odgovarajućih rastvora za razblaženje i bojenje krvnih ćelija. Koncentracija hemoglobina je određena Drabkinovom hemoglobin cijanidskom metodom. Korišten je Drabkinov reagens, pomoću kojeg je hemoglobin preveden u cijanmethemoglobin, čija je ekstinkcija očitana na kolorimetru pri talasnoj dužini od 546 nm. Vrijednost hematokrita je određena postupkom uzimanja krvi u mikrohematokrit cjevčice i centrifugiranjem krvi u mikrohematokrit centrifugi 5 minuta na 16 000 rpm. Krv je uzimana u cjevčice (oko 2/3) koje su prije upotrebe bile heparinisane kako bi se izbjegla koagulacija krvi (Mitrašinović-Brulić i Škrijelj, 2016). Vrijednosti hematoloških indeksa (MCV, MCH, MCHC) utvrđene su uvrštavanjem vrijednosti koncentracije hemoglobina, vrijednosti hematokrita i broja eritrocita u odgovarajuće standardne formule. Određivanje spola vršeno je disekcijom jedinki i posmatranjem gonada, a kod juvenilnih jedinki korištena je lupa. Za statističku obradu podataka korišteni su deskriptivni i analitički statistički parametri 20. verzije SPSS programskog paketa – *IBM SPSS Statistics 20,* kao i programski paket Microsoft Office Excel 2010. Od pokazatelja deskriptivne statistike korišteni su: aritmetička sredina, standardna devijacija, koeficijent varijacije, minimalna vrijednost, maksimalna vrijednost, skjunis, kurtozis. Od parametara analitičke statistike korišteni su analiza varijanse i T-test (Petz i sar., 2012) .

REZULTATI I DISKUSIJA

U tabeli broj 1 prikazane su vrijednosti hematoloških parametara kaliforijske pastrmke analizirani prije i nakon zimskog perioda. U uslovima hipotermije, kod jedinki pastrmke nakon zimskog perioda dolazi do značajnih promjena svih analiziranih hematoloških parametara. Konstatovano je statistički značajno smanjenje vrijednosti hemoglobina, broja eritrocita, MCH i MCHC ($p<0,05$), i statistički značajnog povećanja vrijednosti hematokrita, MCV i broja leukocita ($p<0,05$).

Tabela 1. Hematološki parametri kontrolne i eksperimentalne grupe kalifornijske pastrmke

♂♀ Parametar	Kontrolna grupa (n=40)	Eksperimentalna grupa (n=50)	**T test**
Hb (g/l)	78,99±7,43	42,49±6,71	**p<0,05**
Hct (l/l)	0,39±0,05	0,51±0,13	**p<0,05**
RBC (10^{12}/L)	1,51±1,78	1,32±3,14	**p<0,05**
MCV (fl)	277,67±46,27	350,55±130,72	**p<0,05**
MCH (pg)	53,77±6,81	32,13±9,65	**p<0,05**
MCHC (g/l)	204,51±27,44	86,16±18,05	**p<0,05**
WBC (10^9/L)	2,29±8,07	3,27±7,60	**p<0,05**

Proučavajući analizirane hematološke parametre mužjaka (tabela 2) može se konstatovati da se tokom hipotermije vrijednosti hemoglobina, broja eritrocita, MCH i MCHC smanjuju, dok se vrijednosti hematokrita, MCV i broja leukocita povećavaju. Sve promjene su statistički signifikantne ($p<0,05$). Deskriptivna statistika ukupnog uzorka kontrolne i eksperimentalne grupe riba je predstavljena u prilogu (tabele 1 i 2).

Tabela 2. Hematološki parametri mužjaka kalifornijske pastrmke

♂♂ Parametar	Kontrolna grupa (n=16)	Eksperimentalna grupa (n=24)	**T test**
Hb (g/l)	81,13±6,18	42,26±6,99	**p<0,05**
Hct (l/l)	0,41±0,05	0,56±0,18	**p<0,05**

RBC (10^{12}/L)	1,52±1,89	1,36±2,76	**p<0,05**
MCV (fl)	286,73±57,84	387,36±148,47	**p<0,05**
MCH (pg)	54,13±7,44	32,76±10,26	**p<0,05**
MCHC (g/l)	201,06±30,16	80,50±22,88	**p<0,05**
WBC (10^9/L)	2,33±8,32	3,44±6,87	**p<0,05**

Kod analiziranih ženki (tabela 3.) uočavamo iste promjene kao kod mužjaka. Sve evidentne promjene statistički su značajne (p<0,05).

Tabela 3. Hematološki parametri ženki kalifornijske pastrmke

♀♀ Parametar	Kontrolna grupa (n=24)	Eksperimentalna grupa (n=26)	**T test**
Hb (g/l)	76,54±8,18	42,69±6,68	**p<0,05**
Hct (l/l)	0,37±0,05	0,47±0,07	**p<0,05**
RBC (10^{12}/L)	1,51±1,71	1,28±3,49	**p<0,05**
MCV (fl)	267,32±26,44	318,35±107,51	**p<0,05**
MCH (pg)	53,36±6,25	31,57±9,39	**p<0,05**
MCHC (g/l)	208,45±24,46	91,11±10,96	**p<0,05**
WBC (10^9/L)	2,25±8,06	3,13±8,11	**p<0,05**

Rezultati komparacije hematoloških parametara mužjaka i ženki kalifornijske pastrmke predstavljeni su u tabeli 4., a kompletna statistička obrada u prilogu (tabele 3, 4, 5 i 6). Komparirajući hematološke parametre kontrolne grupe po spolu uočavaju se veće vrijednosti svih parametara u krvi mužjaka osim vrijednosti MCHC. Navedene razlike su statistički značajne samo za vrijednost hemoglobina i hematokrita. Kada se kompariraju prosječne vrijednosti hematoloških parametara po spolu prije i nakon zimskog perioda uočavaju se veće vrijednosti hematokrita, broja eritrocita, MCV, MCH i broja leukocita u krvi mužjaka, dok su veće vrijednosti hemoglobina i MCHC u krvi ženki. Samo se za vrijednost Hct uočavaju statistički značajne razlike. Možemo konstatovati da ženke i kontrolne i eksperimentalne grupe jedinki u odnosu na mužjake, imaju veću vrijednost hemoglobina u litri eritrocita (MCHC) u krvi. Generalno, na osnovu rezultata T – testa, možemo konstatovati da mužjaci i kontrolne i eksperimentalne grupe kalifornijske pastrmke u odnosu na ženke, iako ne statistički značajno (p>0,05) imaju veće vrijednosti analiziranih hematoloških parametara u krvi.

Tabela 4. Analiza spolno specifičnih karakteristika hematoloških parametara kalifornijske pastrmke

Kontrolna grupa (n=40)	N	Hb (g/l)	Hct (l/l)	RBC (10^{12}/L)	MCV (fl)	MCH (pg)	MCHC (g/l)	WBC (10^9/L)
Mužjaci ♂♂	16	81,13	0,41	1,52	286,73	54,13	201,06	2,33
Ženke ♀♀	24	76,54	0,37	1,51	267,32	53,36	208,45	2,25
T test		**p<0,05**	**p<0,05**	p>0,05	p>0,05	p>0,05	p>0,05	p>0,05

Eksperimentalna grupa (n=50)	N	Hb (g/l)	Hct (l/l)	RBC (10^{12}/L)	MCV (fl)	MCH (pg)	MCHC (g/l)	WBC (10^9/L)
Mužjaci ♂♂	24	42,26	0,56	1,36	387,36	32,76	80,50	3,44
Ženke ♀♀	26	42,69	0,47	1,28	318,35	31,57	91,11	3,13
T test		p>0,05	**p<0,05**	p>0,05	p>0,05	p>0,05	p>0,05	p>0,05

Rezultati dosadašnjih istraživanja, a koja se odnose na istraživanja slična našim, pokazuju da se hematološki parametri riba signifikantno razlikuju prije i nakon zimskog perioda uzrokovanom niskom temperaturom. Brojni autori (Angelica D. i sar., 2013; Ciltas A. i sar., 2004; Ivanc A. i sar., 2007) bavili su se istraživanjem utjecaja temperature na hematološke parametre riba.
Treba naglasiti kako je većina autora proučavala utjecaj hipertermije, a manji broj istraživača je proučavao efekte hipotermije. Iako citirani radovi koji se odnose na hipertermiju nisu značajno relevantni za komparaciju sa rezultatima u ovom istraživanju ipak se mogu uzeti u obzir, jer je hipertermija kao i hipotermija, stresni faktor. Pod uticajem stresa izazvanog hipertermijom (Omanović, 2013) kod kalifornijske pastrmke zabilježen je porast vrijednosti Hb (120,74 g/l), RBC (1,607 x 10^{12}/L), MCV (364,81 fl) i WBC (4,917 x 10^9/L), te pad vrijednosti MCHC (206,71 g/l). Jasno je da hipotermija i hipertermija kao jaki stresori izazivaju promjene u hematološkom statusu riba. Kada se kompariraju hipotermija i hipertermija, možemo reći da oba navedena stresora dovode do statistički signifikantnog povećanja MCV i WBC, te do pada vrijednosti MCHC u krvi riba. Prvi odgovor organizma na stres je povećana količina lučenja adrenalina i noradrenalina u krvotok. Adrenalin uzrokuje pojačan rad srca, povećanje brzine cirkulacije iz centralnih organa (izuzev srca i pluća) prema perifernim dijelovima tijela čime se povećava cirkulacija u mozgu i mišićima, gdje pristiže veća količina krvi bogata kisikom, te se povećava frekvencija disanja kako ne bi došlo do hipoksije (Bogut i sar., 2006). Sve navedene karakteristike su moguće u slučaju toplinskog stresa. S obzirom na činjenicu da su ribe bile „zarobljene“ u akvarijumu i da se radi o uzgajanim vrstama pod kontrolisanim uslovima odsutni su određeni procesi koji su u normalnim uslovima prisutni, kao što je migracija. S obzirom da su ribe konstantno izložene fizičko-hemijskim faktorima, fiziološka adaptacija u ovim uslovima se odvija na drugačiji način. U hipertermiji, usljed hipoksije povećava se potreba za O_2, a u hipotermiji ribe miruju, smanjuju fiziološke aktivnosti (npr. prestaju sa hranjenjem) kako bi se „spasile“ energetske rezerve. U ovom slučaju riba nastoji smanjiti gubitak toplote tako da se krv povlači u depoe kako bi se smanjila vazokonstrikcija perifernih sudova i smanjio energetski metabolizam. U tom slučaju smanjuje se eritropoeza. Ovi faktori dovode do stresne reakcije usljed čega se akumuliraju limfociti pa se povećava broj leukocita, a samim time i vrijednost hematokrita. Smanjen broj eritrocita nadomješten je njihovim većim volumenom (a smanjenje vrijednosti Hb i MCH je posljedica ovih reakcija). Hematološki parametri, najčešće vrijednost hematokrita i koncentracija hemoglobina su jako osjetljivi na temperaturne promjene koje se pojavljuju tokom stresa (Mitrašinović i Suljević, 2009). Naša studija pokazuje da kratkotrajna hipotermija

kod jedinki kalifornijske pastrmke dovodi do signifikantnog smanjenja ($p<0,05$) broja eritrocita u krvi. Prema Homatowska i sar. (2002) sa povećanjem temperature, povećava se RBC, Hb i MCHC. Ovaj rezultat proizilazi iz neophodnosti adaptacije na niži sadržaj kisika u vodi. Polazeći od činjenice da smo kod eksperimentalne grupe jedinki zabilježili pad broja eritrocita, naša studija je srazmjerno tome pokazala i statistički signifikantno smanjenje ($p<0,05$) koncentracije hemoglobina u krvi. Zabilježili smo signifikantno smanjenje srednje vrijednosti hemoglobina u litri eritrocita (MCHC) kod jedinki kalifornijske pastrmke tretiranih kratkotrajnom hipotermijom, te signifikantno povećanje ($p<0,05$) broja leukocita (WBC) u krvi eksperimentalne grupe jedinki kalifornijske pastrmke. Davis i sar. (2008) sugerišu da promjene u leukocitnom profilu mogu biti uzrokovane stresom. Očito je da se hipotermija kao jak stresor odrazila na povećanje broja leukocita, kao što smo i očekivali u našoj studiji. Kada su u pitanju vrijednosti hematoloških parametara po spolnoj pripadnosti (Vázquez i Guerrero, 2007) su utvrdili da su hematološki parametri mužjaka i ženki kalifornijske pastrmke jednaki, jedino je vrijednost eritrocita signifikantno različita. Istraživanja, Van Vuren i Hattingh (2006), ukazuju da ne postoji statistički značajna razlika u vrijednostima hematoloških parametara mužjaka i ženki kod iste vrste. Navedene konstatacije su djelomično slične našim istraživanjima. Primjenom t-testa konstatovali smo da mužjaci kalifornijske pastrmke imaju signifikantno veće vrijednosti Hb i Hct, u odnosu na ženke, dok ostali parametri ne pokazuju statističku signifikantnost.

Prema dostupnim literaturnim podacima jasno se vidi da su provedena brojna istraživanja hematoloških osobenosti riba, kako u svijetu, tako i na području Bosne i Hercegovine.

Literaturni podaci o djelovanju hipotermije na hematološki status riba u našoj zemlji su veoma oskudni, te kao smjernicu za buduća istraživanja navodimo ispitivanja efekata hipotermije na hematološke parametre ribljih vrsta na području Bosne i Hercegovine.

ZAKLJUČCI

Na osnovu sprovedenih istraživanja dokazali smo da postoji statistički značajna razlika u vrijednostima hematoloških parametara kalifornijske pastrmke analiziranih prije i nakon zimskog perioda, odnosno nakon hipotermije uzrokovane niskom temperaturom vode u tom periodu.

Efekti hipotermije dovode do značajnih promjena u krvnoj slici kalifornijske pastrmke, gdje su evidentne povećane ili snižene vrijednosti svih analiziranih hematoloških parametara. Konstatovano je statistički značajno povećanje vrijednosti hematokrita, MCV i broja leukocita, te do statistički značajnog smanjenja broja eritrocita, vrijednosti hemoglobina, MCH i MCHC.

Dokazali smo da hipotermija kao jak stresor dovodi do stresne reakcije usljed čega se akumuliraju limfociti pa se povećava broj leukocita po litri krvi. U uslovima hipotermije ribe miruju, smanjuju fiziološke aktivnosti, kako bi se „spasile“

energetske rezerve. U ovom slučaju riba nastoji smanjiti gubitak toplote tako da se krv povlači u depoe kako bi se smanjila vazokonstrikcija perifernih sudova i smanjio energetski metabolizam. U tom slučaju smanjuje se proces eritropoeze. Kada se analiziraju hematološki parametri po spolnoj strukturi možemo konstatovati da mužjaci kalifornijske pastrmke imaju statistički značajno veće vrijednosti hemoglobina i hematokrita u odnosu na ženke kalifornijske pastrmke.

LITERATURA

Angelica D, Cristea V, Lorena D. Influence of thermal stress on the hematological profile of *Oncorhynhus mykiss* held in different stocking densities in recirculating aquaculture systems. Lucrari Scientifice, Seria Zootehnie. 55: 262-272. 2013.

Bogut I, Novoselić D, Pavličević J. Biologija riba, Sveučilište J. J. Strossmayera u Osijeku, Sveučilište u Mostaru. 2006.

Cherniack NS, Oku Y, Saidel GM, Bruce EN, Altose MD. Optimization of breathing through perpeptual mechanisms. In: Takishima T, Cherniack NS (Eds.), Control of Breathing Dyspnea Advances in the Biosciences, Oxford, Pergamon Press. 79: 337–349. 1991.

Ciltas A, Erdogan O, Hisar O, Kocaman EM. Inhibition effect of some chemical anesthetics and hypothermia on the activity of glucose 6-phosphate dehydrogenase from rainbow trout (*Oncorhynchus mykiss*) erythrocytes in vivo. Turkish Journal of Veterinary and Animal Sciences. 28: 813-817. 2004.

Davis AK, Maney DL, Maerz JC. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Functional Ecology. 22: 760-772. 2008.

Dekić R., Ivanc A., Mandić M., Lolić S., Bakrač-Bećiraj A. Utjecaj promjena temperature vode na eritrocitni profil Carassius gibelio. Croatian Journal of Fisheries. 71: 32-36. 2013.

Guyton AC., Hall JE. Medicinska fiziologija, Medicinska naklada, Zagreb. 2003.

Homatowska A., Wojtaszek J., Adamowiez A. Haematological indices and circulating blood picture in the sunbleak, Leucaspius delineatus (Heckel, 1843). Zoologica Poloniae. 47 (3-4): 57-68. 2002.

Ivanc A., Dekić R., Hasković E., Dulčić J., Glamuzina B., Mitrašinović M., Suljević D., Bubić M. The effects of thermal stress on red blood cell parameters of Barbus peloponnesis, ECI XII, XII European Congress of Ichthyology, Cavtat (Dubrovnik). 2007.

Ivanova NT. Atlas Kleotk krovi ryb, "Legkja i iščevaja promyšlennost", Moskva. 1983.

Iversen M., Finstad B., Nilssen KJ. Recovery from loading and transport stress in Atlantic salmon (Salmon salar L.) smolts. Aquaculture. 168: 387-394. 1998.

Kestin SC., Wootton SB., Gregory NG. Effect of slaughter by removal from water on visual evoked activity in the brain and reflex movement of rainbow trout (*Oncorhynchus mykiss*). Veterinary Record. 128: 443-6. 1991.

Kock KH. Antarctic Fish and Fisheries, Cambridge University Press. 231–260. 1992.

McCarthy DH., Stevenson DP., Roberts MS. Some blood parameters of the rainbow trout (*Salmo gairdneri* Richardson), I. The Kamloops variety. Journal of Fish Biology. 5: 1-8. 1973.

Miholjčić M., Jadrić S., Winterhalter-Jadrić M. Biohemija, Svjetlost, Sarajevo. 1988.
Mitrašinović Maja, Suljević D. Hematološki status kljena *Leuciscus cephalus* (Linnaeus, 1758.) iz rijeke Krupice i Željeznice. Veterinaria Sarajevo. 1: 63-76, 2009.
Mitrašinović-Brulić Maja, Škrijelj R. Praktikum iz opšte fiziologije životinja i čovjeka, Univerzitet u Sarajevu, Prirodno-matematički fakultet, Odsjek za biologiju. 2016.
Omanović M. Efekti termičkog stresa na hematološke parametre i procentualnu zastupljenost uobličenih krvnih elemenata kalifornijske pastrmke *Oncorhynchus mykiss* (Walbaum, 1792), Magistarski rad, Univerzitet u Sarajevu. 2013.
Petz B., Kolesarić V., Ivanec D. Petzova statistika: Osnovne statističke metode za nematematičare. Naklada Slap. Zagreb. 2012.
Pörtner HO. Physiological basis of temperature-dependent biogeography: trade-offs in muscle design and performance in polar ectotherms. Journal of Experimental Biology. 205: 2217-2230. 2002.
Selye H. Stress: Eustress, distress and humanperspectives, In: Day, S. (ed), Life stress, Van Nostrand Reinhold, Cincinnati. 1985.
Škrijelj, R. Populacije riba neretvanskih jezera: ihtiološka monografija. Sarajevo: Prirodno-matematički fakultet Univerziteta u Sarajevu, 137 str. 2002.
Škrijelj R., Đug S. Uvod u ekologiju životinja, Prirodno-matematički fakultet, Sarajevo. 2009.
Tseng YC., Liu ST., Hu MY., Chen RD., Lee JR., Hwang PP. Brain functioning under acute hypothermic stress supported by dynamic monocarboxylate utilization and transport in ectothermic fish. Frontiers in Zoology. 11: 53. 2014.
Van Vuren JHJ., Hattingh J. A seasonal study of the haematology of wild freshwater fish. Journal of Fish Biology. 13 (3): 305-313. 2006.
Vázguez GR., Guerrero GA. Characterization of blood cells and hematological parameters in *Cichlasoma dimerus* (Teleostei, Perciformes). Tissue and Cell. 39: 151–160. 2007.
Zorriehzahra MJ., Hassan MD., Gholizadeh M., Saidi AA. Study of some hematological and biochemical parameters of Rainbow trout (*Oncorhynchus mykiss*) fry in western part of Mazandaran province, Iran. Iranian Journal of Fisheries Sciences. 9 (1): 185-198. 2010.

Prilog:

Tabela 1. Hematološki parametri kontrolne grupe kalifornijske pastrmke (n=40)

Analizirani parametri	Hb (g/l)	Hct (l/l)	RBC (10^{12}/L)	MCV (fl)	MCH (pg)	MCHC (g/l)	WBC (10^9/L)
Srednja vrijednost	78,99	0,39	1,51	277,67	53,77	204,51	2,29
Standardna devijacija	7,43	0,05	1,78	46,27	6,81	27,44	8,07
Kurtozis	0,39	-0,97	1,23	6,02	1,57	-0,33	-0,57
Skjunis	-0,34	-0,05	-0,40	1,91	0,98	0,56	0,47
Minimalna vrijednost	63,21	0,30	1,05	213,99	43,55	159,71	1,15
Maksimalna vrijednost	92,72	0,49	1,93	452,38	74,32	261,00	3,95
Koeficijent variranja	9,41	13,06	1,18	16,66	12,66	13,42	2,12

Tabela 2**.** Hematološki parametri eksperimentalne grupe kalifornijske pastrmke (n=50)

Analizirani parametri	Hb (g/l)	Hct (l/l)	RBC (10^{12}/L)	MCV (fl)	MCH (pg)	MCHC (g/l)	WBC (10^9/L)
Srednja vrijednost	42,49	0,51	1,32	350,55	32,13	86,16	3,27
Standardna devijacija	6,71	0,13	3,14	130,72	9,65	18,05	7,60
Kurtozis	-0,89	6,99	2,48	1,21	-0,14	0,57	2,58
Skjunis	0,40	2,22	-0,85	-0,33	0,25	-0,87	1,15
Minimalna vrijednost	32,30	0,37	3,50	43,33	11,66	37,40	1,85
Maksimalna vrijednost	55,60	1,04	2,02	670,97	54,13	112,50	5,65
Koeficijent variranja	15,79	26,25	2,38	37,29	30,03	20,95	2,32

Tabela 3. Hematološki parametri mužjaka kontrolne grupe kalifornijske pastrmke (n=16)

Analizirani parametri	Hb (g/l)	Hct (l/l)	RBC (10^{12}/L)	MCV (fl)	MCH (pg)	MCHC (g/l)	WBC (10^9/L)
Srednja vrijednost	81,13	0,41	1,52	286,73	54,13	201,06	2,33
Standardna devijacija	6,18	0,05	1,89	57,84	7,44	30,16	8,32
Kurtozis	1,54	-1,15	2,71	3,65	2,89	-0,44	-0,79
Skjunis	-0,09	-0,15	-0,45	1,54	1,48	0,53	0,37
Minimalna vrijednost	66,67	0,33	1,05	213,99	43,57	159,71	1,20
Maksimalna vrijednost	92,72	0,49	1,93	452,38	74,32	258,98	3,95
Koeficijent variranja	7,62	12,06	12,46	20,17	13,75	15,00	35,66

Tabela 4. Hematološki parametri mužjaka eksperimentalne grupe kalifornijske pastrmke (n=24)

Analizirani parametri	Hb (g/l)	Hct (l/l)	RBC (10^{12}/L)	MCV (fl)	MCH (pg)	MCHC (g/l)	WBC (10^9/L)
Srednja vrijednost	42,26	0,56	1,36	387,36	32,76	80,50	3,44
Standardna devijacija	6,99	0,18	2,76	148,47	10,26	22,88	6,87
Kurtozis	-1,23	3,34	0,27	1,28	-0,28	-1,07	-0,21
Skjunis	0,11	1,56	-0,81	-0,41	0,69	-0,42	0,47
Minimalna vrijednost	32,30	0,37	7,50	56,00	19,11	37,40	2,30
Maksimalna vrijednost	53,60	1,04	1,72	670,97	54,13	109,52	4,70
Koeficijent variranja	16,53	31,12	20,30	38,33	31,30	28,42	19,97

Tabela 5. Hematološki parametri ženki kontrolne grupe kalifornijske pastrmke (n=24)

Analizirani parametri	Hb (g/l)	Hct (l/l)	RBC (10^{12}/L)	MCV (fl)	MCH (pg)	MCHC (g/l)	WBC (10^9/L)
Srednja vrijednost	76,54	0,37	1,51	267,32	53,36	208,45	2,25
Standardna devijacija	8,18	0,05	1,71	26,44	6,25	24,46	8,06
Kurtozis	-0,08	-1,68	-0,23	0,54	-1,24	0,04	0,11
Skjunis	-0,13	-0,16	-0,40	0,63	0,03	1,03	0,64
Minimalna vrijednost	63,21	0,30	1,15	225,55	43,55	180,28	1,15
Maksimalna vrijednost	91,96	0,43	1,75	326,12	63,86	261,00	3,95
Koeficijent variranja	10,69	12,44	11,33	9,89	11,72	11,74	35,89

Tabela 6. Hematološki parametri ženki eksperimentale grupe kalifornijske pastrmke (n=26)

Analizirani parametri	Hb (g/l)	Hct (l/l)	RBC (10^{12}/L)	MCV (fl)	MCH (pg)	MCHC (g/l)	WBC (10^9/L)
Srednja vrijednost	42,69	0,47	1,28	318,35	31,57	91,11	3,13
Standardna devijacija	6,68	0,07	3,49	107,51	9,39	10,96	8,11
Kurtozis	-0,52	0,11	3,58	1,66	0,11	0,15	6,46
Skjunis	0,74	0,83	-0,80	-1,21	-0,23	0,40	1,88
Minimalna vrijednost	34,10	0,39	3,50	43,33	11,66	72,86	1,85
Maksimalna vrijednost	55,60	0,62	1,02	467,29	46,55	112,50	5,65
Koeficijent variranja	15,64	13,98	27,20	33,77	29,73	12,03	25,96

PRODUKTIVNOST RADA POLUSTACIONARNIH SISTEMA MAŠINSKE MUŽE*

PRODUCTIVITY OF SEMI-STATIONARY MILKING MACHINE SYSTEMS

Selim Škaljić[1], Nermin Rakita[1], Muamer Pekmez[1]

Original scientific paper

Rezime

Savremena poljoprivredna proizvodnja zahtijeva stalno praćenje radnih procesa sa ciljem kontrole i racionalizacije rada. Mašinska muža zauzima značajno mjesto u strukturi rada. Obuhvata procese pripreme muznih grla, pranja vimena, izmuzivanje prvih mlazova, aktivnu mužu, domuzivanje i na kraju pranje izmuzišta. Ako se ima u vidu da se navedeni procesi obavljaju u jutarnjim i večernjim terminima onda se radi o značajnom radnom angažmanu mašinskog i ljudskog rada kojeg stalno treba kontrolisati. Iz navedenog razloga ovo istraživanje je izvedeno na polustacioniranom sistemu mašinske muže koje je u velikom broju zastupljeno na farmama u BiH. Eksperiment je izveden na farmi "PD Butmir d.o.o Sarajevo" čiji kapacitet sa podmlatkom iznosi cca 700 grla. U eksperimentu je odabrano 10 muznih grla na kojima su izvršena hronografska mjerenja utrošenog mašinskog i ljudskog rada u jutarnjoj i večernjoj muži sa osmodnevnim ponavljanjem. Obrađeni rezultati hronografskih mjerenja ljudskog rada ukazuju da 51,4% vremena se odnosi na pranje vimena, 21,5% masaža vimena, 5,5% posušivanje vimena, 8,7% izmuzivanje prvih mlazova, 8,8% postavljanje sisnih čaša i skidanje sisnih čaša 4,1%. Prosječan radni angažman mašinskog rada za 10 ispitivanih muznih grla iznosio je 129,27 min od čega se 77% odnosilo na mašinsku mužu, 19% mašinsko domuzivanje i 4% se odnosilo na zastoje u radu. Po jednom muznom grlu u prosjeku se utrošilo 12,9 minuta mašinskog rada, pri čemu je dnevni prosjek iznosio 15,2 litara mlijeka što ukazuje da se u prosjeku utrošilo 1,84 minuta po namuženom litru mlijeka. Eksperimentom je obuhvaćeno i pranje izmuzišta gdje se u prosjeku utrošilo 113,4 min od čega se na ispiranje instalacije i opreme utrošilo 90 min, a na ostale operacije 23,4 min. U strukturi ostalih operacija kao što je prenošenje muznih jedinica bilo je 32,3%, priključivanje na sistem pranja 22,3% i vanjsko pranje izmuzišta 45,4%. Na temelju ostvarenih rezultata može se konstatovati da muža na polustacioniranim izmuzištima zahtijeva mnogo ljudskog rada i da na većim farmama treba primjenjivati racionalnije sisteme muže.

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina

[1] Poljoprivredno-prehrambeni fakultet Univerziteta u Sarajevu / *Faculty of Agriculture and Food Sciences University of Sarajevo*

Ključne riječi*: muža, mašinski rad, ljudski rad, polustacionarno izmuzište.*

Summary

Modern agricultural production demands continuous monitoring of work processes with purpose of control and rationalization of work. Machine milking takes important role in work structure. Machine milking includes processes of preparation of milking cow, udder cleaning, extra-milking of herds and washing of the milking parlour at the end. Keeping in mind that mentioned processes are performed early in the morning and the evening, it can be said that there is a great amount of machine and human work engagement that needs to be continuously controlled. Because of the above reason, this research is done at semi-stationary milking system which is present at many farms in Bosnia and Herzegovina. The research is conducted at dairy farm "PD Butmir d.o.o." which has the capacity of about 700 cattle, including calves and heifers. In this experiment, ten milking cows have been chosen for taking of chronograph measurements of consumption of human and machine work during the morning and evening milking. Results show that 51.4% of time is spent on udder cleaning, 21.5% for udder massage, 5.5% for udder drying, 8.7% for getting the first stream of milk and 8.8% for attachment and removal of milking cups 4.1%. The average engagement of machinery for milking of ten cows used for the research was 129.27 min, whereas 77% of time was spent on machine milking, 19% on extra-milking and 4% on work interruptions. At average, it amounts to 12.9 min for machine milking per cow. As 15.2 liters of milk were collected per day milking, it means that approximately 1.84 min was spent for each liter of milk. The research also included indicators of washing of milking parlour for which approximately 113.4 min were spent, out of which 90 min were spent on the washing of installations and equipment and 23.4 min for other operations. In structure of other operations, moving of milking units took 32.3%, connecting of the washing system 22.3% and external washing of milking parlour 45.4% of the time. The obtained results show that that milking with semi-portable milking machines requires a lot of human labour and that at larger farms more rational milking systems need to be applied.

Keywords: *milking, machine labour, human labour, semi-stationary milking system*

UVOD

Muža krava je posao koji objedinjuje složene biološke procese i mehaničke principe koje uređaj za mužu treba da zadovoljava. Efikasnost cjelokupnog procesa muže u velikoj mjeri ovisi od optimalno utrošenog mašinskog i ljudskog rada. U brojnim dosadašnjim istraživanjima analiziran je utrošak ljudskog i mašinskog rada u procesu muže. Hansen (1999.) tvrdi da je proces muže najzahtjevniji u većini danskih farmi. Chang i sar. (1992.) navode kako na mužu se troši preko 50% dnevnog angažmana radnog osoblja u SAD-u. Schmidt i Johnston (1997.) došli su do istih pokazatelja i ukazuju da se to odnosi na preko 40% farmi u Australiji. Prema istraživanju Armstrong (1994.) unaprjeđenje efikasnosti procesa muže ima značajan uticaj na povećanje broja muznih krava na farmama. Imajući u vidu navedene pokazatelje cilj ovog istraživanja je da primjenom hronografske metode mjerenja utvrdimo utrošak vremena ljudskog i mašinskog rada kod polustacioniranih sistema muže, koji su u velikom broju prisutni u BiH. Također želi se utvrditi struktura rada po fazama muže, prosječan utrošak dnevnog angažmana po muznom grlu i jednom litru namuženog mlijeka a sve u cilju postizanja veće efikasnosti.

MATERIJAL I METODE RADA

Eksperimentalna istraživanja izvedena su na farmi "PD Butmir d.o.o Sarajevo" čiji kapacitet sa podmlatkom iznosi cca 700 grla. Usljed dužine trajanja samog ispitivanja, metodom slučajnog uzorka, odabrano je 10 grla koja se nalaze u vezanom sistemu držanja. Metod na kome se zasniva istraživanje obuhvatio je hronografska mjerenja operacija u procesu mašinske muže. Radi se o polustacioniranom sistemu muže gdje se proces muže obavlja na ležištu. Mlijeko se pomoću sistema mljekovoda doprema do laktofriza i čuva do trenutka odvoza u mljekaru. Proces muže se odvija u dva navrata tokom dana (ujutro i navečer), te je u svrhu dobivanja preciznih podataka o utrošenom mašinskom i ljudskom radu praćena samo jedna muzna jedinica. Radi lakšeg praćenja ukupan ljudski rad razvrstan je u radne procese koji obuhvataju: pranje vimena, posušivanje vimena, masažu vimena, izmuzivanje prvih mlazova mlijeka, postavljanje sisnih čaša i skidanje sisnih čaša. Sa druge strane, ukupan mašinski rad tokom muže obuhvata procese aktivne muže i domuzivanja. Istraživanjem je obuhvaćeno vrijeme utrošeno na pranje muznih jedinica, mljekovodne instalacije i postrojenja u širem smislu. Rezultati dobivenih mjerenja obrađeni su metodom deskriptivne statistike, te iskazani kroz vremenski angažman mašinskog i ljudskog rada po jednoj muznoj kravi i litri namuženog mlijeka.

REZULTATI I DISKUSIJA

Rezultati provedenog istraživanja ukazuju da se za mužu deset krava, prosječne mliječnosti 15,2 litara/dan, u prosjeku utrošilo 267,48 minuta ukupnog dnevnog vremena. U strukturi tog vremena priprema krava iznosila je 9%, muža 46%, ispiranje postrojenja 34% i ostale operacije 11%. Angažovani ljudski rad utrošen za pripremu muže iznosio je 23,37 minuta, skidanje sisnih čaša 0,99 minuta što je ukupno iznosilo 24,36 min/dan, dok je prosječan dnevni angažman mašinskog rada sa uračunatim zastojima iznosio 129,7 min/dan. Rezultati mjerenja ukazuju da je veći angažman mašinskog i ljudskog rada zabilježen tokom jutarnje muže u odnosu na večernju, uslijed veće količine namuženog mlijeka. Ukupan prikaz utrošenog rada pri mašinskoj muži, dat je u tabeli 1.

Tabela 1. Utrošeni rad u procesu mašinske muže.
Table 1. Work in the process of machine milking.

Faze muže	Operacije	Prosječne vrijednosti trajanja muže				Utrošeno po litru
		Jutarnja	**Večernja**	**Ukupno**	**Po kravi**	
		(min.)	(min.)	(min.)	(min.)	(min./L)
A. Priprema	Pranje vimena	9,34	3,20	12,54	1,25	-
	Masaža vimena	3,21	2,03	5,24	0,52	-
	Izmuzivanje prvih mlazova	1,23	0,88	2,11	0,21	-
	Posušivanje	0,70	0,63	1,33	0,13	-
	Postavljanje sisnih čaša	1,14	1,00	2,15	0,21	-
	Ukupno priprema:	**15,63**	**7,74**	**23,37**	**2,34**	-
B. Muža	Aktivna muža	56,91	42,55	99,46	9,95	1,48
	Domuzivanje	13,68	11,49	25,17	2,52	0,36
	Ukupno muža:	**70,59**	**54,04**	**124,64**	**12,46**	**1,84**
C. Ostalo	Skidanje čaša	0,51	0,48	0,99	0,09	-
	Pranje opreme	58,20	55,22	113,42	11,34	-
	Gubici vremena	2,37	2,70	5,06	0,51	-
	Ukupno muža:	**61,08**	**58,40**	**119,47**	**11,94**	-
Sveukupno (A+B+C):		**147,30**	**120,18**	**267,48**	**26,74**	-

Obrađeni rezultati odnose se na 10 muznih krava čija prosječne mliječnosti u osmodnevnom mjerenju je iznosila 15,2 litra/dan mlijeka. Deskriptivni statistički pokazatelji za dnevni utrošak mašinskog i ljudskog rada mogu se vidjeti u nastavku.

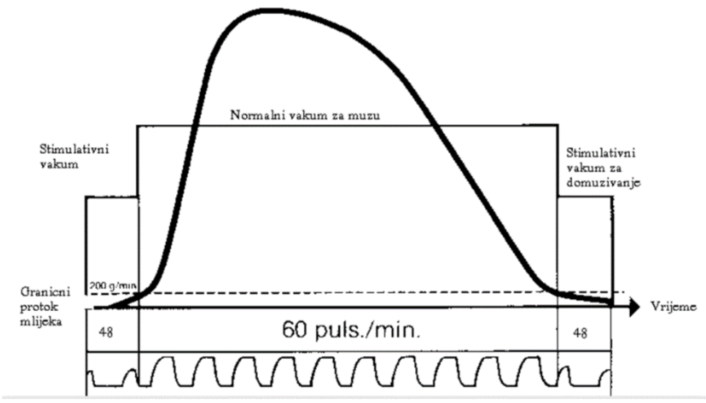
Tabela 2. Statistički pokazatelji utrošenog rada na dan.
Table 2. Statistical indicators of spent work per day.

Ukupne dnevne aktivnosti (min)	Deskriptivni statistički pokazatelji				
	s.v.	**Max**	**Med**	**Min**	**s.d.**
Pranje vimena (PV)	12,54	14,17	13,09	8,83	1,74
Posušivanje (P)	1,33	1,36	1,33	1,28	0,03
Masaža vimena (MV)	5,24	6,30	5,20	4,30	0,64
Izmuzivanje prvih mlazova (IPM)	2,11	2,68	2,33	1,00	0,61
Postavljanje sisnih čaša (PSČ)	2,15	2,43	2,03	1,98	0,19

Aktivna muža (AM)	99,46	127,33	96,53	79,15	16,15
Domuzivanje (D)	25,17	27,67	25,00	22,50	1,99
Skidanje čaša (SČ)	0,99	1,02	1,00	0,93	0,03
Pranje opreme (PO)	113,42	116,05	113,35	112,00	1,40
Gubici vremena (GV)	5,06	7,69	4,58	2,85	1,67

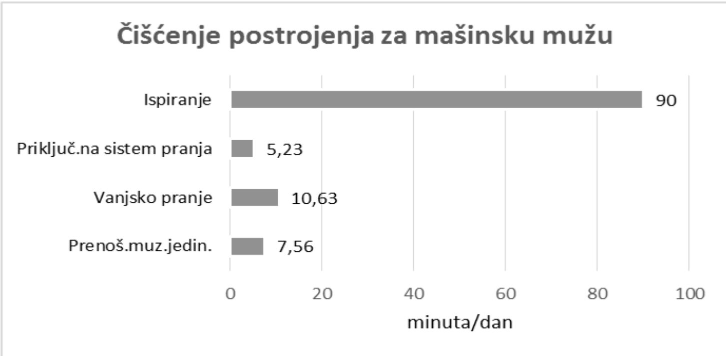
Legenda: s.v. - srednja vrijednost; Max - maksimum; Med - medijan; Min - minimum; s.d. - standardna devijacija.

Iz obrađenih rezultata može se vidjeti da su hronografska mjerenja bila ujednačena i da nema ekstremnih odstupanja. Analiza strukture ljudskog rada u procesu muže (24,36 min/dan) pokazala je da najveći procenat utrošenog vremena otpada na pranje vimena 51,5%, zatim 21,5% masažu vimena, postavljanje sisnih čaša 8,8%, izmuzivanje prvih mlazova mlijeka 8,7% i 4,1% skidanje sisnih čaša nakon završene muže. U navedenim procentima nije uključen ljudski rad za pranje opreme, koji zaslužuje zasebnu analizu. Analiza strukture mašinskog rada (219,7 min/dan) pokazala je da je na aktivnu mužu odlazilo 99,46 min (45%), domuzivanje 25,17 min (12%), ispiranje instalacije 90 min (41%) i zastoje u radu 5,06 min (2%). U odnosu na ukupan dnevni rad (267,48 min) mašinski rad iznosio je čak 82,14% a ljudski 17,86%. Prosječna vrijednost utrošenog mašinskog rada po litri namuženog mlijeka, iznosila je 1,84 min. Proces domuzivanja u jutarnjoj muži trajao je duže po jednoj kravi i iznosio je 1,36 min, dok je večernje domuzivanje zbog manje produkcije mlijeka bilo kraće i iznosilo 1,14 min. Domuzivanje je bilo mašinsko sa „*Duovak“*pulzatorima koji imaju promjenjivi broj pulzacija, što zaslužuje dodatnu analizu efikasnosti. Iz narednog grafikona se vidi krivulja protoka mlijeka gdje se muža najvećim dijelom odvija pri normalnom vakuumu od 60 pulzacija u minuti.



Graf. 1. Protok mlijeka kod pulzatora sa promjenjivim brojem pulzacija.
Graph. 1. Milk flow in a pulse with a variable number of pulsations.

Kod smanjenog protoka mlijeka uključivao se mehanizam «*Duovak*» koji je smanjivao broj pulzacija, a produžavao amplitude. Ekonomski efekat domuzivanja mogu se vidjeti iz količine namuženog mlijeka koji je iznosio 0,91 litar po kravi ili 6% od ukupno namuženog mlijeka. Održavanje higijene i pranje postrojenja za mašinsku mužu zauzimalo je značajan radni angažman. Struktura utrošenog mašinskog i ljudskog rada u procesu pranja može se vidjeti u sljedećem prikazu.



Graf. 2. Struktura mašinskog i ljudskog rada u procesu pranja uređaja.
Graph. 2. Structure of machine and human work in the process of washing.

Pranje uređaja za mašinsku mužu dnevno zahtijeva 113,42 minuta. Od ukupno utrošenog vremena za pranje, najviše zauzima ispiranje instalacije mljekovoda 90 min (79%), vanjsko pranje 10,63 min (9%), zatim prenošenje muznih jedinica 7,56 min (7%) i priključenje na sistem pranja 5,23 min (5%). U odnosu na ukupan dnevno utrošeni rad, na pranje se utrošilo 42,4%.

Uspoređivanje ostvarenih rezultata sa istraživanjima drugih autora, Mijić i sar. (2003) dalo je slične pokazatelje. Najveći dio angažmana ljudskog rada činile su operacije vezane za pripremu vimena i pranje. Iste rezultate potvrdio je Hansen (2000.) pri čemu je dodao da muža čini između 55 i 78% ukupnog rada na farmi. Iz navedenog razloga, optimizacija procesa muže sa osvrtom na poboljšanje efikasnosti rada na farmi je od iznimne važnosti. Nielsen i Sorensen (1993.) navode kako je u odnosu na slobodni sistem držanja, u vezanom sistemu potreban mnogo veći angažman ljudskog rada za pripremu vimena i stavljanje sisnih čaša, te da je čišćenje izmuzišta nakon muže iznimno zahtjevan posao. Prema istraživanju Stahla i sar. (1999.) zbog visokog utroška ljudskog rada za farme sa preko 70 muznih grla nije efikasno koristiti polustacionarni sistem izmuzišta. Iste konstatacije navodi i Hansen (2000.). Sa druge strane Nielsen i Sorensen (1993.) navode kako stacionarni sistem izmuzišta može biti efikasan za farme sa manjim brojem grla. Slične navode iznose Gustafsson i Lindquist (1987.) da polustacionarni sistemi izmuzišta imaju manju efikasnost u odnosu na stacionarne sisteme. Kao glavni razlog, navodi se činjenica da je udaljenost između životinja tokom muže u stacionarnom sistemu muže znatno manja i da je to jedan od razloga veće efikasnosti ljudskog rada. Kod polustacioniranih sistema muže pored smanjene efikasnosti ljudskog rada, javljaju se negativne posljedice po zdravlje radnika. Naime, prema istraživanju pomenutih autora, nepravilan položaja kičmenog stuba tokom obavljanja radnih operacija može dovesti do zdravstvenih problema.

ZAKLJUČAK

Dobiveni rezultati ispitivanja jutarnje i večernje muže kod polustacionarnog sistema ukazuju da se dnevno na jednu kravu prosječne mliječnosti 15,2 litara utrošilo 26,75 min/dan ukupnog mašinskog i ljudskog rada. Na proces pripreme krave utrošilo se 2,34 min po grlu ili 8,74% ukupnog vremena, na mužu i domuzivanje 12,46 min ili 46,61% i ostale operacije koje uključuju skidanje muznih čaša, pranje i zastoje u radu 11,95 minuta ili 44,64% ukupnog rada. Analiza navedenih pokazatelja ukazuje na sljedeće:

- Priprema krava za mužu (2,34 min/krava) obuhvatila je pranje vimena (51,4%), masaža vimena (21,5%), posušivanje vimena (5,5%), izmuzivanje prvih mlazova (8,7%), postavljanje sisnih čaša (8,8%) i skidanje sisnih čaša (4,1%).
- Na aktivnu dnevnu mužu odnosilo se 9,95 min/krava (79,85%) i domuzivanje 2,52 min/krava (20,15%), što ukupno iznosi u dvokratnoj muži 12,46 min/krava.
- Ostale operacije su uključivale skidanje muznih čaša (0,75%), zastoji u radu (4,25%) i pranje postrojenja za mužu (95,00%), što je ukupno iznosilo u dvokratnoj muži 11,94 min/krava.

Na temelju dobivenih rezultata zaključuje se da polustacionirani sistemi mašinske muže imaju veliko učešće mašinskog i ljudskog rada po jednom muznom grlu. Veliki utrošak vremena na prenošenju muznih jedinica, pranje i masažu vimena te ispiranje centralnog mljekovoda ukazuju da polustacionirani sistemi muže mogu se primjenjivati na farmama sa manjim brojem životinja, što ukazuju i prikupljena literaturna istraživanja, a da kod većih farmi prednost treba davati stacionarnim postrojenjima muže.

LITERATURA

Armstrong, D. V., Smith, J. F., Gamroth, M. J. and Bucklin, R. (1994): Milking parlour performance in the United States. Dairy systems for the 21st century, Proceedings of the 3rd International Dairy Housing Conference, Orlando, 2-5 February 1994, 59 –69.

Chang, W., Barry, M. C., Jones, L. R. and Merrill, W. G. (1992): Compares computerised milking parlour evaluation system. Journal of Dairy Science Vol. 75, 578-586.

Gustafson, B. & Lundquist, P. (1987): Working postures and human health problems in Swedish milk production. Latest development in livestock housing.

Proceedings of the Seminar of the 2nd Technical Section of the CIGR, 118–126.

Hansen, M. N. (2000): Comparison of the labour requirement involved in the housing of dairy cows in different housing systems. *Acta Agriculture Scandinavia, Section A-Animal Science*, *50*(3), 153-160.

Hansen, M. N. (1999): Optimal number of clusters per milker *Journal of Agricultural Engineering Research* 72: 341-346.

Mijić, P., Knežević, I., Kuterovac, K., Dakić, A. (2003). Povezanost krivulje protoka mlijeka i broja somatskih stanica u mlijeku simentalskih krava. Stočarstvo: Časopis za unapređenje stočarstva, 57(1), 13-20. Preuzeto s http://hrcak.srce.hr/178445

Nielsen, V. & Sorensen, C. G. (1993): 'DRIFT'. 'Technical Farm Management', a program for calculation of work requirement, work capacity, work budget and work profile. Danish Institute of Agricultural Engineering, Report No. 53. (In Danish.)

Schmidt W. and Johnston, M. (1997): Benchmarking labour productivity on Victorian dairy farms. *Ellinbank Annual Report 95/96-97/97*, 5-46.

Stahl, T. J., Conlin, B. J., Seykora, A. J., & Steuernagel, G. R. (1999): Characteristics of Minnesota Dairy Farms that Significantly Increased Milk Production from 1989-19931, 2. *Journal of dairy science*, *82*(1), 45-51.

FAKTORI BOJE I SENZORNI KVALITET KOBASICA SARAJEVSKE I KREŠEVKE*

COLOUR FACTORS AND SENSORY QUALITY OF SARAJEVSKA AND KREŠEVKA SAUSAGES

Sabina Operta[1]**, Katarina Budimir[1], Velida Smailhodžić[1], Jasmina Tahmaz[1]

Orginal scientific paper

Rezime

Ovaj rad fokusiran je na određivanje parametara boje, sadržaja soli i natrij nitrita, te senzornog kvaliteta dvije slične fermentirane kobasice Sarajevska i Kreševka koje su proizvedene u istim kontroliranim uslovima. Goveđe meso i loj za Sarajevsku kobasicu usitnjeni su na promjer 6 mm, a za Kreševku na promjer 4 mm. Pored dodataka, koji su isti za obje kobasice, u Sarajevsku je dodat ekstrakt crvene paprike, a u kobasicu Kreševka, bijeli luk. Rezultati istraživanja pokazali su da su postojale značajne razlike ($p<0,05$) u vrijednostima boje (L*, a* i b*), sadržaju rezidualnih nitrita, teksturi, mirisu i ukupnom senzornom kvalitetu. Kreševka je bila svjetlija, crvenija i imala je manji sadržaj rezidualnih nitrita (L* 54,02; a* 11,76; $NaNO_2$ 7,75 mg/kg) u odnosu na Sarajevsku kobasicu (L* 42,54; a* 8,59; $NaNO_2$ 9,80 mg/kg). Sarajevska kobasica je bila žuća (b* 14,73) u odnosu na Kreševku (b* 10,15). Sadržaj soli (3,71%; 3,80%) činio je obje kobasice umjereno slanim. Senzorni kvalitet kobasice Sarajevska bio je bolji u odnosu na kobasicu Kreševka. Kod obje kobasice izgled je ocijenjen kao „dobar“, te je bio najbolje ocijenjeno svojstvo. Tekstura, izgled i boja presjeka bili su „prihvatljivi“ kod obje kobasice. Najlošije ocijenjena senzorna svojstva bili su miris i aroma koji su dobili ocjene „zadovoljavajuće“. Ukupan senzorni kvalitet kobasice Sarajevska iznosio je 64,13% a kobasice Kreševka 60,46%. Boljoj ocjeni senzornog kvaliteta Sarajevske kobasice doprinijele su krupnije čestice masti na presjeku i prihvatljivija boja zbog dodatka ekstrakta crvene paprike. U budućoj proizvodnji kobasica Sarajevske i Kreševke, radi postizanja boljeg mirisa i arome, preporučuje se promjena začina u kombi smjesi.

Ključne riječi: *fermentirane kobasice, L*, a*, b*, $NaNO_2$; NaCl, senzorni kvalitet*

---

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
**Corresponding author: sabinaoperta@yahoo.com
[1]Faculty of Agriculture and Food Science University of Sarajevo, Bosnia and Herzegovina

Summary

This paper is focused on determining the parameters of colour, salt and sodium nitrite content, and on the sensory quality of two similar fermented sausages (Sarajevska and Kreševka) that are produced in the same controlled conditions. Beef meat and fat for the Sarajevska sausage are cut up in small pieces to the diameter of 6 mm and for Kreševka to diameter of 4 mm. In addition to supplements that are same for both sausages, extract of red pepper was added in Sarajevska while garlic was supplemented in Kreševka. The results of the research show that there were significant differences ($p < 0.05$) in the colour values (L*, a* and b*), the content of residual nitrites, texture, smell and overall quality. Kreševka was brighter, redder and had a lower content of residual nitrites (L* 54.02, a* 11.76; NaNO2 7.75 mg/kg) in relation to the Sarajevska sausage (L* 42.54, a* 8, 59; NaNO2 9.80 mg/kg). Sarajevska was yellower (b* 14.73) in relation to Kreševka sausage (b* 10.15). The salt content (3.71%, 3.80%) made both sausages moderately salty. Sarajevska had better sensory quality than Kreševka. In both sausages, appearance was evaluated as "good" and was the best rated property. The texture, appearance and colour of cut section were "acceptable" for both sausages. The smell and flavour were the worst evaluated sensory properties with "satisfactory" evaluation. Total sensory quality of Sarajevska was 64.13% and 60.46% for Kreševka. Larger particles of fat on the cut section and more acceptable colour because of the addition of the red pepper extract contributed to the better sensory quality evaluation of Sarajevska sausage. In the future production of Sarajevska and Kreševka sausages a change of spices is recommended in order to achieve better smell and aroma.

Key words: *fermented sausages, L*, a*, b*, NaNO2; NaCl, sensory quality*

UVOD

Boja fermentiranih kobasica je veoma važan parametar koji utiče na potrošačku prihvatljivost proizvoda. Naročito za ove kobasice važna je boja presjeka. Boja mišićnog tkiva potiče od pigmenta mioglobina. Što je veći sadržaj mioglobina, boja mišićnog tkiva je tamnija. Pored mišićnog tkiva, na presjeku fermentiranih kobasica, a s obzirom da se radi o grubo usitnjenoj sirovini, vidljive su i čestice masnog i vezivnog tkiva. Boja masnog tkiva je bijela do žuta, što zavisi vrste životinje od koje potiče. Na boju mesnih proizvoda, pored sadržaja mioglobina, utiču i drugi faktori. Tako Toldra (2007) navodi da boja mesnih proizvoda zavisi i od: vlažnosti, sadržaja masti i proteina, stanja mioglobina, odnosno njegovoj povezanosti sa okolišem. Boja je pod uticajem pada i krajnje pH vrijednosti, a može biti i pod uticajem prisutnih začina kao što je crvena paprika. Višak kiseline, nastao djelovanjem laktobacila, može isto uticati na boju fermentiranih kobasica. Karakteristična boja nastaje kao reakcija nitrita s mioglobinom. Nitriti se reduciraju na azot oksid, a potom azot oksid djeluje s mioglobinom pri čemu nastaje NO-mioglobin koji daje kobasici karakterističnu ružičasto-crvenu boju (Scott i Hui, 2004). Uloga nitrita, kao dodatka u suhomesnate proizvode, prvenstveno je stvaranje stabilne ružičasto-crvene boje mesa, inhibicija rasta bakterija, razvoj arome te prevencija lipidne oksidacije. Prema Anonimus (2008) rezidualnih nitrita ne smije biti više od 50 mg/kg. Na boju mesnih proizvoda djeluje i sama so. Ukoliko se so koristi sama, mesni proizvodi postaju suhi, tvrdi i smeđi, zbog formiranja metmioglobina (L u, 2010). Iz tog razloga, so se koristi sa nitratima/nitritima te šećerima/sladilima, a uobičajeno se dodaje u rasponu od 2% do 4%, ovisno o tehnologiji i zahtjevima tržišta. Previše dodane soli će rezultirati jako slanim konačnim proizvodom, a s druge strane, premalo soli negativno utiče na očuvanje proizvoda. Zbog toga je jako važno utvrditi optimalnu količinu soli koja će se dodati u nadjev fermentiranih kobasica.

Na senzorni kvalitet fermentiranih kobasica djeluje veliki broj činilaca, a najznačajniji su: vrsta i kvalitet upotrebljenog mesa i masnog tkiva, izbor i količina dodataka, način izrade, aktivnost prisutne endogene mikroflore, brzina i intenzitet procesa dimljenja i sušenja, odnosno klimatski/ambijentalni uslovi sredine (temperatura, relativna vlažnost i cirkulacija zraka), te dužina i stepen zrenja (proteoliza i lipoliza) (Vuković, 2006; Roseiro i dr., 2008; Petrović i dr., 2011). Glavni činioci prihvatanja suhih fermentiranih kobasica su boja, tekstura i aroma.

Cilj ovoga rada je ispitati parametre boje, soli, natrij nitrita i senzornog kvaliteta kobasica Sarajevske i Kreševke, te utvrditi da li postoje značajne razlike između dvije kobasice u ovim parametrima.

MATERIJAL I METODE RADA

Proizvodnja kobasica

Dva tipa komercijalnih, fermentiranih kobasica (Sarajevska i Kreševka) su proizvedena u MIS Stanić u Kreševu (BiH) od po 10 kg. Goveđe meso i goveđi loj usitnili su se na promjer 6 mm (Sarajevska) i 4 mm (Kreševka). Potom, osnovnoj sirovini dodati su: nitritna so (2,5%), kombi smjesa (začini, laktoza, dekstroza, maltodekstrin, aroma pšenica i soje, pojačivači okusa E621, E627, E631, kuhinjska so, ekstrakti začina s celerom, antioksidans E 316, aroma dima) i starter kulture. Pored navedenih dodataka u Kreševku je dodan bijeli luk, a u Sarajevsku kobasicu ekstrakt crvene paprike. Gotov nadjev punjen je na punilici pod vakumom, u kolagene omotače (Ø55). Obje kobasice, tokom 30 dana, podvrgnute su istim kontrolisanim uslovima kondicioniranja (17 °C; <60% RH; 9 sati), fermentacije (22 °C; 90-92% RH; 0,5-0,8 m/s; 2 dana), dimljenja (21 °C; 80-85% RH; 0,2-0,5 m/s; 5 dana/20 minuta/dan), sušenja i zrenja (21-17 °C; 85-75% RH; 0,1-0,2 m/s; 22 dana).

Metode

Sadržaj nitrita determinisan je metodom po Grea i Mirna-u, a sadržaj NaCl-a metodom po Mohru. Boja kobasica (CIE L*, a* i b* vrijednosti) mjerena je pomoću kolorimetra

(Minolta Chroma Meter, model CR-400, Osaka, Japan) na mjernom području (D65/10°, C/2°) od 8 mm.

Senzorna analiza – Kobasice su senzorno ocjenjivane na kraju procesa proizvodnje, a ocjenjivani su slijedeći parametri: izgled, izgled presjeka, boja presjeka, tekstura, miris i aroma te ukupan kvalitet, primjenom 5-bodovne strukturne skale (1 = veoma loš, 2 = zadovoljavajući, 3 = prihvatljiv, 4 = dobar i 5 = izvrstan). Ukupan kvalitet se dobio primjenom slijedeće formule: *UK (%) = 2 x izgled + 4 x izgled presjeka + 3 x boja presjeka + 4 x tekstura + 2 x miris + 5 x okus*

Statistička analiza – Podaci su analizirani korištenjem statističkog paketa SPSS verzija 20 (SPSS inc. Chicago, IL, USA). Studentov t-test korišten je za određivanje značajnih razlika ($p<0,05$) između dvije kobasice. Za provjeru međuzavisnosti (korelacije) između pojedinih parametara koristio se Pearsonov koeficijent korelacije.

REZULTATI RADA I DISKUSIJA

Boja - Kobasica Kreševka bila je značajno ($p<0,05$) svjetlija (L*) u odnosu na Sarajevsku kobasicu. Vrijednosti a* i b* su se također značajno ($p<0,05$) razlikovale između ove dvije kobasice, s tim što je crvenilo (a*) bilo izraženije kod Kreševke, a žutilo (b*) kod Sarajevske kobasice. Visoka vrijednost L* kod kobasice Kreševka (54,02) može se objasniti visokim nivom oksidacije masti. Nam i Ahn (2003) u svojim istraživanjima takođe navode visoku vrijednost L* koja za usitnjenu govedinu ide preko 52. Dobivena prosječna vrijednost L* Sarajevske kobasice je manja od vrijednosti koje su dobili Üren i Babayigit (1997) prilikom ispitivanja boje turskih fermentiranih kobasica (49,6) dok je kod kobasice Kreševka vrijednost L* mnogo veća. Mnogo niže vrijednosti L*, navode Bozkurt i Bayram (2006) te Habtamu (2012) u ispitivanju goveđih kobasica. Vrijednost a* tj. crvenilo bila je značajno manja kod Sarajevske kobasice (8,59) u odnosu na Kreševku (11,76). Manje crvenilo (a*) Sarajevske kobasice može se objasniti dodatkom ekstrakta crvene paprike, jer ekstrakt crvene paprike daje svijetlu crvenu boju. Üren i Babayigit (1997) navode raspon a* od 11,3 do 20,4 iz čega slijedi da se kobasica Kreševka nalazi unutar ovoga raspona, a Sarajevska kobasica ne. Bozkurt i Bayram (2006) su dobili niže vrijednosti a* (7,9), a Habtamu (2012) pak veće (12,68). Tokom prvih dana zrenja azotni spojevi prisutni u mesu, u kombinaciji s mioglobinom proizvode željeni pigment boje. Ovaj pigment ima crvenu boju, odnosno a-vrijednost je povećana. Poslije, do kraja procesa zrenja, dolazi do denaturacije formiranog pigmenta tako da se a-vrijednost smanjuje (Bozkurt i Bayram, 2006). Značajno veću vrijednost b* (žutila) imala je Sarajevska kobasica (14,73) u odnosu na Kreševku (10,15). Vrijednost b* Sarajevske kobasice je bila u rasponu vrijednosti (11,5-26,2) koju su dobili i Üren i Babayigit (1997). Mnogo manju vrijednost b* (7,7) su dobili Bozkurt i Bayram (2006) koji objašnjavaju ovu pojavu na način da potrošnja kisika od strane mikroorganizama i smanjenje oksimioglobina doprinose žućoj boji kobasica.

NaCl - Sadržaj NaCl-a u ispitivanim kobasicama (3,71% i 3,80%) je bio optimalan što ih je prilikom konzumiranja činilo umjereno slanim. Gasparik-Reichardt i dr. (2005) su u istraživanju tradicionalnih fermentiranih kobasica porijeklom iz BiH, zabilježili dosta veći sadržaj NaCl-a od 4,32%. Operta i dr. (2007) navode sličan prosječni sadržaj soli u bosanskom sudžuku (3,31%) s varijacijama od 3,10 do 3,65%. Papadima i dr. (1999) za grčke tradicionalne kobasice navode da sadrže 2,36% - 4,13% NaCl-a, a rezultati sadržaja NaCl-a u kobasicama Sarajevska i Kreševka kreću se u ovom rasponu. Veće vrijednosti sadržaja soli su dobili Tupajić (1991), Operta (2008), Operta i Smajić (2012) i Operta i dr. (2012) u istraživanju bosanskog sudžuka, dok su izuzetno visoke vrijednosti sadržaja soli (8,33%), dobili Operta i dr. (2008) za sudžuk iz domaćinstva. Comi i dr. (2005) u istraživanjima prirodnih talijanskih kobasica navode da je u tri različite fermentacije sadržaj NaCl-a bio 3,37%, 3,37% i 3,28% što pokazuje da su manje slane od kobasica Sarajevske i Kreševke.

Nitriti - Konačna vrijednost rezidualnih nitrita u Sarajevskoj kobasici prosječno je iznosila 7,79 mg/kg, dok je Kreševka imala značajno ($p<0,05$) viši sadržaj NO_2 (9,80 mg/kg). Dobijene razlike u sadržaju rezidualnih nitrita mogu biti posljedica neujednačene zastupljenosti nitritne soli u nadjevu kobasica, stalnom prelasku nitrita u oblik nitrate i obrnuto, količini dostupnog mioglobina za vezivanje, kao i stepenu masnoće u proizvodu. Sadržaj rezidualnih nitrita u obje fermentirane kobasice je ispunjavao uslove Anonimus (2008). Sadržaj rezidualnih nitrita u Sarajevskoj kobasici kretao se u rasponu 3,05-9,46 mg/kg koje navode mnogi autori (Gajić, 2000; Operta i Smajić, 2012; Operta i dr., 2013; Operta i dr., 2015) za slične bosanske, goveđe, fermentirane kobasice, kao i Comi i dr. (2005) u istraživanjima prirodnih fermentiranih talijanskih kobasica (8,83-8,67 ppm). Malo veće vrijednosti od navedenih imala je kobasica Kreševka.

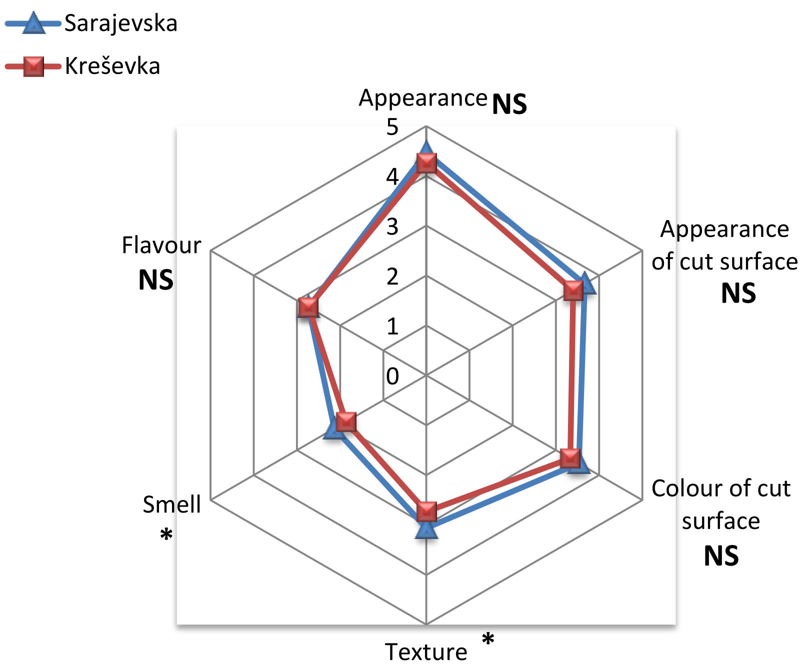
Senzorni kvalitet - Rezultati senzorne analize kobasica Sarajevske i Kreševke predstavljeni su u Tabeli 1 i na Slici 1. Komisija za senzoriku nije detektovala značajne razlike u izgledu, izgledu i boji presjeka i u aromi. Vanjski izgled obje kobasice bio je prihvatljiv jer se omotač lahko skidao, a površina je bila ujednačena i glatka. Sarajevska kobasica imala je prihvatljiviji izgled presjeka zbog boljeg izgleda i rasporeda mišićnog i masnog tkiva i zbog veće granulacije čestica. Kod Kreševke bilo je primjetno razmazivanje uslijed spajanja sitnih čestica masnoće. Sarajevska kobasica imala je nešto višu prosječnu ocjenu za svojstvo boje presjeka zbog vidljivog prisustva ekstrakta crvene paprike te bjelje boje masnog tkiva u odnosu na Kreševku, kod koje je boja masnog tkiva bila zamućena. Tekstura Sarajevske kobasice bila je prihvatljiva u odnosu na Kreševku, a razlog je vjerovatno manji sadržaj masnoće u ovoj kobasici. Veći sadržaj masnoće u kobasici Kreševka, prilikom usitnjavanja u ustima činio je ovu kobasicu masnijom i ljepljivom, te manje prihvatljivom od strane ocjenjivača. Najniže ocjene su dodijeljene mirisu. To bi se moglo objasniti posljedicom veoma dominantnog i čudnog mirisa, nespecifičnog za goveđe fermentirane kobasice. Netipični miris je najvjerojatnije poticao od nekog začina, no kako začini nisu pojedinačno navedeni u upotrebljenoj kombi smjesi, nije utvrđeno o kojem začinu se radi. Kao i kod mirisa, aroma kobasica Sarajevska i Kreševka je bila nespecifična za goveđe fermentirane kobasice, odnosno, nije se osjetila uobičajena aroma fermentiranih kobasica na goveđe meso, dim, bijeli luk, biber i slično. Ukupan kvalitet bio je nešto bolji kod Sarajevske u odnosu na kobasicu Kreševka. Generalno se može reći da je na prihvatljivu teksturu, sočnost, aromu i izgled presjeka vjerovatno veliki uticaj imao visok sadržaj masti (koji se mogao vizualno uočiti na presjeku, te se mogao procijeniti na osnovu razmazivosti na presjeku i u ustima), jer kako navodi Wirth (1988), masnoća pridonosi aromi, teksturi u ustima, mazivosti, sočnosti i nježnosti koji određuju senzorni kvalitet i prihvatljivost suhih kobasica. Također, Papadima i Bloukas (1999) su izvijestili da su kobasice sa 30% masti imale najmanje ocjene za izgled i konzistenciju, bile su svjetlije boje i veoma mekane zbog visokog sadržaja masti. Izuzetno jako negativna Pirsonova korelacija utvrđena je između izgleda presjeka i sadržaja rezidualnih nitrita ($r = -1,0$; $p<0,01$), ali i izuzetno jako pozitivna između izgleda presjeka i vrijednosti b* ($r = 0,99$; $p<0,040$) kod kobasice Kreševka. To znači da je izgled presjeka bio bolje ocijenjen ako je kobasica imala manji sadržaj nitrita i veću vrijednost b*, i obrnuto.

Tabela 1. Fizikalno-hemijski i senzorni parametri kvaliteta kobasica Sarajevske i Kreševke

Table 1. Physicochemical and sensory properties of sausages Sarajevska and Kreševka

Svojstva / Properties	Sarajevska	Kreševka	Statistički značajne razlike / Statistically significant differences
	$\overline{x}$ ± SD	$\overline{x}$ ± SD	
Svjetloća (L*) / Lightness (L*)	42,54 ± 1,11	54,02 ± 1,62	*
Crvenilo (a*) / Redness	8,59 ± 0,73	11,76 ± 0,93	*
Žutilo (b*) / Yellowness	14,73 ± 0,73	10,15 ± 0,74	*
Sadržaj NaCl / NaCl content (%)	3,71 ± 0,17	3,80 ± 0,11	NS
Sadržaj $NaNO_2$ / $NaNO_2$ content (mg/kg)	7,79 ± 0,18	9,80 ± 0,20	*
Ukupni kvalitet/Overall quality	64,13 ± 0,83	60,46 ± 0,30	*

(*Significantly different ; NS- Not significant)



Slika 1. Senzorni parametri kobasica Sarajevske i kreševke

Figure 1. Sensory properties of sausages Sarajevska and Kreševka

(*Significantly different ; NS- Not significant)

ZAKLJUČAK

Visoka vrijednost L* kod kobasice Kreševka može biti posljedica oksidativnih procesa na mastima. Po sadržaju soli, obje kobasice su umjereno slane. Sadržaj rezidualnih nitrita u ispitivanim kobasicama ispunjavao je uslove pravilnika. Boljoj ocjeni senzornog kvaliteta Sarajevske kobasice doprinijele su krupnije čestice masti na presjeku i prihvatljivija boja zbog dodatka ekstrakta crvene paprike. U budućoj proizvodnji kobasica Sarajevske i Kreševke, radi postizanja boljeg mirisa i arome, preporučuje se promjena začina u kombi smjesi.

LITERATURA

Anonimus (2008): Pravilnik o uslovima upotrebe prehrambenih aditiva u hrani namijenjenoj za ishranu ljudi. Službeni glasnik BiH, broj 83.

Bozkurt, H., Bayram, M. (2006): Colour and textural attributes of sucuk during ripening. Meat science. 73 (2): 344 - 350.

Comi, G., Urso, R., Iacumin, L., Rantsiou, K., Cattaneo, P., Cantoni, C., Cocolin, L. (2005): Characterisation of naturally fermented sausages produced in the North East of Italy. Meat Sci. 69:381-392.

Gajić, B. (2000): Kontaminiranost suhomesnatih proizvoda supstancama štetnim po zdravlje ljudi. Magistarski rad. Poljoprivredni fakultet Sarajevo.

Gaspraik - Reichardt, J., Toth, Sz., Cocolin, L., Comi, G., Drosinos, E., Cvrtila, Z., Kozačinski, L., Smajlović, A., Saičić, S., Borović, B. (2005): Technological, physicochemical and microbiological characteristics of traditionally fermented sausages in Mediterranean and central European countries. Tehnologija mesa 46 (3-4): 143 - 153.

Habtamu, A. (2012): Development and evaluation of chickpea and rice based dry fermented sausages. Master Thesis, Addis Ababa Institute of Technology, School of Graduate Studies, Ethiopia.improve color, lipid oxidation, and volatiles of irradiated raw and cooked turkey breast

Lu, Y. (2010): The development of a cured, fermented sheepmeat sausage designed to minimise species and pastoral-diet flavours. Master Thesis, AUT University, New Zealand.

Nam, KC., Ahn, DU. (2003): Use of double packaging and antioxidant combinations to improve color, lipid oxidation, and volatiles of irradiated raw and cooked turkey breast patties. Poult Sci. 82 (5): 850-7.

Operta S., Leko N., Tahmaz J., Alkić M. (2015): Ispitivanje fizikalno-kemijskih i senzornih svojstava „Vlašićke“ kobasice tijekom čuvanja (Investigation of hysicochemical and sensory properties of „Vlašićka“ sausage during storage). Časopis Meso, Vol. XVII, broj 3, str. 247-253, pp 272-278.

Operta S., Tahmaz J., Alić B. (2013): The influence of raw material on physical-chemical properties of Bosnian sudžuk with addition of starter cultures. Proceedings –24th International scientific-expert conference on agriculture and food industry, Sarajevo, September 25–28. 2013. Str. 270-274.

Operta, S. (2008): Kvalitet bosanskog sudžuka porijeklom iz komunalne klaonice. Radovi Poljoprivredno-prehrambenog fakulteta Univerziteta u Sarajevu, Vol. LIII br. 59/1 20. Sarajevo. Str. 209 - 217.

Operta, S., Dževdetbegović, M., Čorbo, S., Tahmaz, J., Šehović, A. (2012): Fizičko-hemijska i senzorna svojstva bosanskog sudžuka proizvedenog u kontrolisanim uslovima od svežeg ohlađenog i zamrznutog mesa. Tehnologija mesa Vol. 53, No. 2, Beograd. Str: 148 - 156.

Operta, S., Smajić, A. (2012): Hemijski parametri kvaliteta bosanskog sudžuka proizvedenog optimiziranjem tradicionalnog postupka proizvodnje u klasičnoj pušnici. Radovi Poljoprivredno-prehrambenog fakulteta Univerziteta u Sarajevu, Vol. LVII br. 62/2. Sarajevo. Str: 103 - 122.

Operta, S., Smajić, A., Ganić, A. (2007): Kvalitet bosanskog sudžuka proizvedenog u industrijskim uslovima. Radovi Poljoprivredno-prehrambenog fakulteta Univerziteta u Sarajevu, Vol. LII br. 58/1. Sarajevo. Str: 239 - 247.

Operta, S., Smajić, A., Ganić, A., Karahmet, E. (2008): Tehnologija i kvalitet bosanskog sudžuka porijeklom iz domaćinstva. Radovi Poljoprivredno-prehrambenog fakulteta Univerziteta u Sarajevu, Vol. LII br. 58/1. Sarajevo. Str. 209-217

Papadima S. N., Bloukas J. G. (1999): Effect of fat level and storage condicions on quality characteristics og tradicional Greek sausages. Meat Sci., 51. 103-113.
Papadima, S. N., Arvanitoyannis, I., Bloukas, J. G., Fournitzis, G. C. (1999): Chemometric model for describing Greek tradicional sausages. Meat Sci 51: 271-277.
Petrović, Lj., Džinić, N., Ikonić, P., Tasić, T., Tomović, V. (2011): Quality and safety standardization of traditional fermented sausages. Tehnologija mesa, 2, 234-244.
Roseiro, L. C., Santos, C., Sol, M., Borges, M. J., Anjos, M., Gonçalves, H., Carvalho, A. S. (2008): Proteolysis in Painho de Portalegre dry fermented sausage in relation to ripening time and salt content. Meat Science, 79 (4), 784-794.
Scott, S. J. and Hui, Y. H. (2004): Food Processing Principles and Applications. Victoria: Blackwell Publishing.
Toldra, F. (2007): Handbook of Fermented Meat and Poultry. Oxford OX4 2DQ, UK: Blackwell Publishing Ltd.
Tupajić, P. (1991): Tehnologija proizvodnje pršuta i sudžuka od goveđeg mesa. Magistarski rad. Poljoprivredni fakultet Sarajevo.
Üren, A., Babayigit, D. (1997): Colour parameters of Turkish-type fermented sausage during fermentation and ripening. Meat Science, 45, 539 - 549.
Vuković I. K. (2006): Osnove tehnologije mesa, Treće izmenjeno i dopunjeno izdanje, Veterinarska komora Srbije, Beograd.
Wirth, F. (1988): Technologies for making fat-reduced meat products. Fleischwirtsch, 68 (9), 1153-1156.

KARAKTERIZACIJA KOBASICA SARAJEVSKA I KREŠEVKA S ASPEKTA NUTRITIVNE VRIJEDNOSTI I STABILNOSTI ZA SKLADIŠTENJE*

CHARACTERIZATION OF SARAJEVSKA AND KREŠEVKA SAUSAGES FROM ASPECT OF NUTRITIONAL VALUE AND STABILITY FOR STORAGE

Sabina Operta[1]**, Katarina Budimir[1], Emsad Keran[2]

Orginal scientific paper

Rezime

Cilj istraživanja bio je karakterizacija dvije kobasice Sarajevske i Kreševke s aspekta nutritivne vrijednosti i parametara stabilnost (pH i aw-vrijednosti). Analize kobasica rađene su prije punjenja u omotače, trećeg, desetog, dvadesetog i tridesetog dana (finalni proizvod) procesa proizvodnje. Analiza varijanse pokazala je značajan uticaj procesa fermentacije i sušenja na pH, aw-vrijednost i gubitak vlage (kalo) kod obje kobasice. Na kraju procesa proizvodnje između Sarajevske i kobasice Kreševka nije bilo velikih razlika (p>0,05) u aw-vrijednosti (0,816; 0,815), kalu (37,96%; 33,98%), sadržaju vode (21,05%; 21, 00%), masti (41,84%, 44,39%) ukupnih proteina (28,90%; 28,58%) i pepela (4,84%, 4,95%). Razlike (p<0,05) su postojale u pH-vrijednosti (4,85; 4,74) i sadržaju proteina vezivnog tkiva (12,99%; 11,59%). Nizak sadržaj vode, pH-vrijednost <4,85 i aw-vrijednost <0,816 svrstavaju kobasice Sarajevsku i Kreševku u brzo fermentirane, suhe goveđe kobasice stabilne za skladištenje nakon 30 dana. Također, ove kobasice su nutritivno vrijedne jer imaju visok sadržaj ukupnih proteina i relativno povoljan sadržaj proteina vezivnog tkiva. Najveći nedostatak istraživanih kobasica je visok sadržaj masnoće, koji treba reducirati u budućoj proizvodnji.

Ključne riječi: *fermentirane goveđe kobasice, karakterizacija, fizikalno-hemijski parametri*

Summary

The aim of the research was to characterize two sausages, Sarajevska and Kreševka in terms of nutritional value and parameter stability (pH and aw-value). Sausage analysis was performed before loading into the wrappers on the third, tenth, twentieth and thirtieth day (final product) of the production process. The variance analysis showed a

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
**Corresponding author: sabinaoperta@yahoo.com
[1] Faculty of Agriculture and Food Science University of Sarajevo, Bosnia and Herzegovina
[2] Meat industry Stanić, Kreševo, Bosnia and Herzegovina

significant influence of fermentation and drying on pH, aw-value and loss of moisture (loss of weight) of both sausages. At the end of the production process there were no significant differences between Sarajevska and Kreševka sausages ($p > 0.05$) in aw-value (0.816, 0.815), los of weight (37.96%, 33.98%), water content (21.05% 21,00%), fat (41.84%, 44.39%), total protein content (28,90%, 28,58%) and ash (4.84%, 4.95%). Differences ($p < 0.05$) existed in pH values (4.85, 4.74) and in connective tissue protein content (12.99%, 11.59%). Low water content, with pH value <4.85 and aw-value <0.816 classifies sausages Sarajevska and Kreševka into fast fermented dry beef sausages stable for storage after 30 days. Also, these sausages are nutritionally valuable because they have a high content of total proteins and a relatively favorable content of the connective tissue protein. The biggest disadvantage of the researched sausages is high fat content, which should be reduced in future production.

Key words: *fermented beef sausage, characterization, physico-chemical properties*

UVOD

Goveđe fermentirane kobasice proizvode se od mješavine usitnjenog čistog goveđeg mesa i goveđeg loja uz dodatak soli, šećera, nitrita i/ili nitrata, začina i često starter kultura. Mješavina ili nadjev puni se u prirodna crijeva ili kolagene omotače, a potom podvrgava procesima fermentacije, sušenja i zrenja s ili bez dimljenja. Usljed fizikalno-biohemijskih reakcija u kobasicama i djelovanju spoljašnjih uslova (temperatura, protok i stepen relativne vlažnosti) tokom ovih procesa, dolazi do formiranja fizikalno-hemijskih, senzornih i mikrobioloških svojstava finalne kobasice. Tokom proizvodnog procesa dešavaju se mnogobrojne promjene kao što su: pad pH-vrijednosti, promjene u početnoj mikroflori, reduciranje nitrata u nitrite, a kasnije u azot oksid, formiranje nitrozomioglobina, solubilizacija i gelifikacija miofibrilarnih i sarkoplazmatskih proteina, proteolotičke i lipolitičke promjene i dehidracija (Casaburi i dr., 2007). Sigurnost finalne kobasice postiže se kombinacijom različitih faktora uključujući: dodatu so, antimikrobni metabolizam, prisustvo aditiva (nitriti, nitrati), nisku pH i aw-vrijednost (Messens i dr., 2003). Na kraju procesa proizvodnje suhe fermentirane kobasice su stabilne za skladištenje ukoliko imaju pH<0,52 i/ili aw-vrijednost <0,91. U propisima većine evropskih zemalja za vrjednovanje kvaliteta fermentiranih kobasica pored sadržaja proteina, kolagena i masti, uzimaju se u obzir pH i Aw-vrijednost. No, u Bosni i Hercegovini većina fermentiranih kobasica prema Pravilniku o usitnjenom mesu, poluproizvodima i proizvodima od mesa (Službeni glasnik BiH, 82/13; Anonimus, 2013) vrjednuju se samo po sadržaju ukupnih proteina i proteina vezivnog tkiva. U tipu goveđih, fermentiranih kobasica koje se proizvode po proizvođačkoj specifikaciji, na području Bosne i Hercegovine, pored bosanskog sudžuka, u manjem obimu proizvode se i druge kobasice kao što su Sarajevska, Kreševka, Vlašićka, goveđi kulen, čajna kobasica, itd. Po upotrijebljenoj osnovnoj sirovini i dodacima kobasice Sarajevska i

Kreševka jako su slične. Razlika se odnosi na usitnjenost, tako da se meso i masnoća za Sarajevsku kobasicu usitnjavaju na promjer 6 mm, a za kobasicu Kreševka na 4 mm. U obje kobasice dodaju se isti dodaci, s tim da se u Sarajevsku dodaje još i ekstrakt crvene paprike, a u Kreševku bijeli luk. Obje kobasice ni na koji način nisu do sada istraživane.
Cilj ovih istraživanja je karakterizacija dvije veoma slične goveđe, fermentirane kobasice s aspekta nutritivne vrijednosti i parametara stabilnosti na kraju proizvodnog postupka.

MATERIJAL I METODE RADA

Proizvodnja kobasica

Dva tipa komercijalnih, fermentiranih kobasica (Sarajevska i Kreševka) proizvedena su u mesnoj industriji Stanić u Kreševu (BiH). Goveđe meso i goveđi loj usitnili su se na promjer 6 mm (Sarajevska) i 4 mm (Kreševka). Osnovnoj sirovini dodati su: nitritna so (2,5%), kombi smjesa (začini, laktoza, dekstroza, maltodekstrin, aroma pšenica i soje, pojačivači okusa E621, E627, E631, kuhinjska so, ekstrakti začina s celerom, antioksidans E 316, aroma dima) i starter kulture. Pored navedenih dodataka u Kreševku dodan je bijeli luk, a u Sarajevsku kobasicu ekstrakt crvene paprike. Gotov nadjev punjen je na punilici pod vakumom, u kolagene omotače (Ø55). Narednih 30 dana obje kobasice, podvrgnute su istim kontrolisanim uslovima kondicioniranja (17 °C; <60% RH; 9 sati), fermentacije (22 °C; 90-92% RH; 0,5-0,8 m/s; 2 dana), dimljenja (21 °C; 80-85% RH; 0,2-0,5 m/s; 5 dana/20 minuta/dan), sušenja i zrenja (21-17 °C; 85-75% RH; 0,1-0,2 m/s; 22 dana).

Metode

Fizikalno-hemijske analize: Uzorci za analize uzeti su poslije punjenja a prije fermentacije (0. dan), poslije fermentacije (3. dan), tokom sušenja (10. i 20. dan) i na kraju procesa proizvodnje (30. dan). pH-vrijednost mjerenja je pH-metrom sa ubodnom elektrodom (Eutech Instruments, Netherlands) a Aw-vrijednost, Aw-metrom (LabSwift-aw, Novasina, Switzerland). Gubitak mase (kalo) tokom sušenja izračunat je na osnovu odnosa finalne i početne mase pomnoženo sa 100. Određivanje sadržaja vode, ukupnih proteina, masti, hidroksiprolina i pepela rađeno je po standardima BAS ISO (2007) metoda. Sadržaj kolagena izračunat je na osnovu množenja sadržaja hidroksiprolina (%) sa faktorom 8. Relativan sadržaj vezivno-tkivnih proteina izračunat je iz odnosa sadržaja kolagena (%) i ukupnih proteina (%) pomnoženo sa 100. *Statistička analiza*: Dobijeni podaci obrađeni su kroz analizu varijanse koristeći statistički program SPSS, verzija 20 (SPSS inc. Chicago, IL, USA). Značajne razlike testirane su Tukey testom ($p<0,05$). Studentov *t*-test korišten je za određivanje značajnih razlika ($p<0,05$) u ispitivanim parametrima između dvije kobasice.

REZULTATI RADA I DISKUSIJA

Parametri stabilnosti (pH i aw-vrijednost)

Tokom fermentacije došlo je do značajnog pada pH-vrijednosti kod obje kobasice (Sarajevska = s 5,45 na 4,80; Kreševka = s 5,47 na 4,79). Pad pH-vrijednosti posljedica je djelovanja odabranih strater kultura, koje su razložile šećere na mliječnu kiselinu. Na pad pH-vrijednosti utiče i hidroliza masti pri kojoj nastaju slobodne masne kiseline. Do kraja procesa proizvodnje pH-vrijednost kobasice Sarajevska povećala se neznatno (4,84), a kod kobasice Kreševka neznatno smanjila (4,74). Prema Rödel i dr. (1993) povećanje pH-vrijednosti može biti rezultat stvaranja amonijaka usljed razgradnje aminokiselina. Slične rezultate pH-vrijednosti dobili su Operta i dr. (2013) za bosanski sudžuk sa dodatkom starter kultura (4,8-4,9) i Operta i dr. (2015) za bosanski sudžuk i kobasicu Vlašićka (4,84-4,81). S obzirom na pH-vrijednost i ove kobasice su jako kisele, te se može potvrditi činjenica koju iznose Gasparik – Reichardt i dr. (2005) da su tradicionalne fermentirane kobasice (4,86) iz BiH jedne od najkiselijih. Aw-vrijednost ispitivanih kobasica, konstantno i značajno ($p<0,05$) opadala je tokom tehnološkog procesa proizvodnje, te je za obje kobasice na kraju proizvodnje iznosila oko 0,815. Postupno smanjivanje Aw-vrijednosti u kobasici tokom proizvodnje bio je rezultat dehidratacije (Čavlek i Mavračić, 1993), odnosno postupnog isparavanja vode i difuzije soli u kobasicu tokom sušenja (Lorenzo, 2014). Kobasice Sarajevska i Kreševka mogu se smatrati stabilnim za skladištenje s aspekta pH i aw-vrijednosti jer ispunjavaju kriterije (pH≤5,0 ili Aw≤0,91) prema Leistner i Roedel (1975).

Tabela 1. Promjene pH, aw-vrijednosti i kala tokom fermentacije, sušenja i zrenja
Table 1. Changes of pH, aw-value and weight loss during fermentation, drying and ripening

Sarajevska	Dani/Days				
Parametri/ Properties	0. dan/day	3. dan/day	10. dan/day	20. dan/day	30. dan/day
pH-value	$5,45^{a}$ ±0,00	$4,80^{c}$ ±0,01	$4,81^{c}$ ±0,02	$4,88^{b}$ ±0,00	$4,84^{bc}$ ±0,00
Aw-value	$0,958^{a}$ ±0,00	$0,930^{b}$ ±0,00	$0,893^{c}$ ±0,00	$0,847^{d}$ ±0,00	$0,816^{e}$ ±0,00
Weight loss (%)	-	$16,22^{d}$ ±0,37	$27,94^{c}$ ±0,37	$33,89^{b}$ ±0,37	$37,96^{a}$ ±2,51
Kreševka					
Parametri/ Properties	0. dan/day	3. dan/day	10. dan/day	20. dan/day	30. dan/day
pH-value	$5,47^{a}$ ±0,00	$4,79^{b}$ ±0,00	$4,74^{c}$ ±0,03	$4,75^{bc}$ ±0,00	$4,74^{c}$ ±0,01
Aw-value	$0,942^{a}$ ±0,00	$0,918^{b}$ ±0,00	$0,886^{c}$ ±0,00	$0,836^{d}$ ±0,00	$0,816^{e}$ ±0,00
Weight loss (%)	-	$14,28^{d}$ ±0,15	$25,18^{c}$ ±0,32	$31,27^{b}$ ±0,30	$33,98^{a}$ ±0,21

Means within same rows with different letters (a-e) are significantly different ($p<0,05$).

Nutritivna vrijednost

Tokom postupka proizvodnje došlo je do postepenog gubitka vlage, čime se povećavalo kalo kobasica. Gubitak mase fermentiranih kobasica tokom proizvodnog procesa nastaje kao posljedica sušenja proizvoda, odnosno isparavanja vlage iz proizvoda. Veće kalo kod Sarajevske kobasice može se objasniti kao posljedica veće granulacije osnovnih sastojaka (promjer 6 mm) i manjeg sadržaja masti u odnosu na Kreševku (promjer 4 mm, veći sadržaj masti). Također, smanjenje pH-vrijednosti ima uticaj na povećano isparavanje vode tokom fermentacije i procesa sušenja (Rabi i dr., 2006). Operta i dr. (2012; 2015) za slične goveđe kobasice navode visoko kalo slično Sarajevskoj kobasici. Mnogo veće kalo za sudžuk (47,09%) u svojim istraživanjima navode Čaušević i dr. (1985). Na kraju postupka proizvodnje usljed velikog gubitka vlage došlo je do povećane koncentracije masti, proteina i pepela kod obje kobasice, bez značajnih razlika (p>0,05) u njihovom sadržaju između kobasica. Značajne razlike (p<0,05) utvrđene su jedino za relativni sadržaj vezivnog tkiva (kolagen/ukupni proteini mesa pomnoženo sa 100). Sadržaj vode obje kobasice kretao se u rasponu od 18,90% do 28,80% koji za bosanski sudžuk navode Operta i dr. (2007; 2015). U istraživanjima Papadima i dr. (1999) za grčke tradicionalne kobasice prosječan sadržaj vode je mnogo veći (43,98%).

Sadržaj masti u kobasicama Sarajevska i Kreševka izrazito je visok i kretao se u rasponu 36,21%-49,84%, koji za komercijalno proizveden bosanski sudžuk u svojim istraživanjima navode Operta i dr. (2007; 2015). Komercijalne kobasice na početku proizvodnje imaju oko 32% masti, ali tokom sušenja postotak se poveća na 40-50% (Wirth, 1988). Niži sadržaj masti navode Papadima i dr. (1999) za grčke tradicionalne kobasice (prosječno 33,50%) i Comi i dr. (2005) u istraživanjima prirodnih fermentiranih talijanskih kobasica (27,70% - 35,80%). Siriken i dr. (2009) za turski sucuk navode da sadrži od 29% do 42% masti iz čega se zaključuje da se dobivena vrijednost sadržaja masti u Sarajevskoj kobasici ipak nalazi unutar raspona navedenih vrijednosti, dok kobasica Kreševka ima veći sadržaj masti.

Po sadržaju ukupnih proteina (>28%) i proteina vezivnog tkiva (<13%) kobasice Sarajevska i Kreševka su nutritivno vrijedne kobasice koje ispunjavaju uslove pravilnika (Anonimus, 2013) u pogledu sadržaja ovih materija. Odnos vlaga:proteini (M:P) kod Sarajevske kobasice je 0,72:1, a kod Kreševke 0,73:1 čime ispunjavaju preporuke FSIS (USDA, 2010) za stabilno uskladištene kobasice koje moraju imati odnos M:P od 1,9:1 ili manje. Sadržaj proteina u Sarajevskoj i Kreševki sličan je sadržaju proteina sudžuka (25,50% do 33,50%) u istraživanjima Operta i dr. (2007; 2012; 2013), te Vlašićke kobasice sa prosječno 26,60% masti u istraživanju Operta i dr. (2015). Papadima i dr. (1999) navode manji sadržaj proteina za grčke tradicionalne kobasice (prosječno 19,19 %), kao i Comi i dr. (2005) u istraživanjima prirodnih fermentiranih talijanskih kobasica (19,13-21,67%). Proteini su u nutritivnom i tehnološkom pogledu najvredniji sastojci proizvoda od mesa. Samim time, sadržaj proteina predstavlja objektivan kriterij na osnovu kojeg se može vrjednovati kvalitet proizvoda (Vuković, 2001).

ZAKLJUČAK

Nizak sadržaj vode (oko 21%), pH-vrijednost manja od 4,85 i aw manja od 0,816 pokazuje da su kobasice Sarajevska i Kreševka brzo fermentirane suhe goveđe kobasice stabilne za skladištenje nakon 30 dana. Također, ove kobasice su nutritivno vrijedne jer imaju visok sadržaj ukupnih proteina i relativno povoljan sadržaj proteina vezivnog tkiva. Najveći nedostatak Sarajevske i Kreševke kobasice je izrazito visok sadržaj masnoće, koji u budućoj proizvodnji treba reducirati kako bi se postigao bolji kvalitet ovih kobasica.

Tabela 2. Hemijski pokazatelji kvaliteta kobasica Sarajevske i Kreševke
Table 2. Chemical properties of sausages Sarajevska and Kreševka

Parametri/ Properties (%)	Sarajevska $\bar{X} \pm SD$	Kreševka $\bar{X} \pm SD$	Statistički značajne razlike / Statistically significant differences
0. dan/day			
Sadržaj vlage/ Moisture content	51,36 ± 0,46	48,88 ± 1,91	NS
Sadržaj masti/ Fat content	30,52 ± 0,57	32,12 ± 1,57	NS
Ukupni proteini/ Total proteins	13,30 ± 0,16	13,40 ± 0,17	NS
Sadržaj pepela/ Ash content	3,36 ± 0,23	3,62 ± 0,24	NS
Sadržaj kolagena/UP X 100; Collagen content/ TP X 100	27,80 ± 0,39	22,14 ± 0,40	*
30. dan/day			
Sadržaj vlage/ Moisture content	21,05 ± 2,51	21,00 ± 1,00	NS
Sadržaj masti/ Fat content	41,84 ± 2,20	44,39 ± 1,03	NS
Ukupni proteini/ Total proteins	28,90 ± 0,59	28,58 ± 0,35	NS
Sadržaj pepela/ Ash content	4,84 ± 0,03	4,95 ± 0,15	NS
Sadržaj kolagena/UP X 100; Collagen content/ TP X 100	12,99 ± 0,22	11,59 ± 0,19	*

NS – not significant; *p<0,05 ; UP - Ukupni proteini; TP - Total proteins

LITERATURA

Anonimus (2013): Pravilnik o usitnjenom mesu, poluproizvodima i proizvodima od mesa. Službeni glasnik BiH, broj: 82/13.

Casaburi A., Aristoy M-C., Cavella S., Di Monaco R., Ercolini D., Toldra´ F., Villani F. (2007). Biochemical and sensory characteristics of traditional fermented sausages of Vallo di Diano (Southern Italy) as affected by the use of starter cultures. Meat Science 76, 295-307.

Comi, G., Urso, R., Iacumin, L., Rantsiou, K., Cattaneo, P., Cantoni, C., Cocolin, L. (2005): Characterisation of naturally fermented sausages produced in the North East of Italy. Meat Sci. 69:381-392.

Čaušević, Z., Milanović, A., Glogovac, Ž., Lelek, M. (1985): Prilog poznavanju proizvodnje sudžuka. Radovi poljoprivrednog fakulteta. Poljoprivredni fakultet Univerziteta u Sarajevu.

Čavlek, B., Mavračić, Z. (1993): Utjecaj dodataka na proces zrenja fermentiranih kobasica, Prehrambeno-tehnološka biotehnićka revija 31 (1), 6-57.

Gasparik-Reichardt, J., Toth, Sz., Cocolin, L., Comi, G., Drosinos, E., Cvrtila, Z., Kozačinski, L., Smajlović, A., Saičić, S., Borović, B. (2005): Technological, physicochemical and microbiological characteristics of traditionally fermented sausages in Mediterranean and central European countries. Tehnologija mesa 46 (3-4): 143-153.

Leistner, L., Roedel, W. (1975): The significance of water activity for microorganisms in meats. In Duckworth, R.B., (eds. 9 Water Relation in Foods) pp 309-323. Academic Press, London

Lorenzo, M. (2014). Changes on physico-chemical, textural, lipolysis and volatile compounds during themanufacture of dry-cured foal "cecina". Meat Science, 96, 256–263. Vandendriessche, Frank (2008). Meat products in the past, today and in the future. Meat

Messens, W., Verluyten, J., Leroy, F., & De Vuyst, L. (2003). Modelling growth and bacteriocin production by Lactobacillus curvatus LTH 1174 in response to temperature and pH values used for European sausage fermentation processes. International Journal of Food Microbiology, *81*(1), 41-52.

Operta S., Leko N., Tahmaz J., Alkić M. (2015). Ispitivanje fizikalno-kemijskih i senzornih svojstava „Vlašićke" kobasice tijekom čuvanja (Investigation of hysicochemical and sensory properties of „Vlašićka" sausage during storage). Časopis Meso, Vol. XVII, broj 3, str. 247-253, pp 272-278.

Operta S., Tahmaz J., Alić B. (2013): The influence of raw material on physical-chemical properties of Bosnian sudžuk with addition of starter cultures. Proceedings –24th International scientific-expert conference on agriculture and food industry, Sarajevo, September 25–28. 2013. Str. 270-274.

Operta, S., Dževdetbegović, M., Čorbo, S., Tahmaz, J., Šehović, A. (2012). Fizičko-hemijska i senzorna svojstva bosanskog sudžuka proizvedenog u kontrolisanim uslovima od svežeg ohlađenog i zamrznutog mesa. Tehnologija mesa Vol. 53, No. 2, Beograd. Str: 148-156.

Operta, S., Smajić, A., Ganić, A. (2007): Kvalitet bosanskog sudžuka proizvedenog u industrijskim uslovima. Radovi Poljoprivredno-prehrambenog fakulteta Univerziteta u Sarajevu, Vol. LII br. 58/1. Sarajevo. Str: 239-247.

Papadima, S. N., Arvanitoyannis, I., Bloukas, J. G., Fournitzis, G. C. (1999): Chemometric model for describing Greek tradicional sausages. Meat Sci 51: 271-277.

Rabi, S. M., Banani, R. C., Runu, C., Utpal, R. C. (2006): Effect of fermentation and drying temperature on the characteristics of goat meat (Black Bengal variety) dry sausage. African Journal of Biotechnology, 5(16): 1499-1504.

Rödel, W., Stiebing, A., Kröckel, L. (1993): Ripening parameters for traditional dry sausages with a modul covering. Fleischwirtschaft 73: 848-853. Science, 78, 104-113.

Siriken, B., Cadirci, O., Inat, G., Yenisey, C. (2009): Some Microbiological and PysicioChemical Quality of Turkish Sucuk (Sausage). Journal of Animal and Veterinary Advences. 8. (10): 2027-2032.

USDA. (2010). Principles of preservation of shelf-stable dried meatproducts. Preuzeto: 10.05.2013. godine sa: http://origin-www.fsis.usda.gov/PDF/FSRE_SS_7Principles. pdf.

Vuković, I.K. 2001: Važniji činioci i kriterijumi kvaliteta proizvoda od mesa - Prilozi za novi Pravilnik o kvalitetu. Tehnologija mesa 46 (5-6): 421-432. Beograd.

Wirth, F. (1988). Technologies for making fat-reduced meat products. Fleischwirtsch, 68,1153-1156.

UTICAJ KUHANJA NA TEŽINU, SADRŽAJ VODE I AW-VRIJEDNOST PILEĆIH HRENOVKI

THE EFFECT OF COOKING ON WEIGHT, WATER CONTENT AND AW-VALUE OF CHICKEN HOT DOGS

Sabina Operta[1], Vedada Emšo[1], Jasmina Tahmaz[1], Nermina Đulančić[1]

Original scientific paper

Rezime

U ovom istraživanju analizirana je deklaracija, težina, sadržaj vode i aw-vrijednost pilećih hrenovki šest različitih domaćih proizvođača. Domaći proizvođači pilećih hrenovki ne ispunjavaju uslove deklarisanja u pogledu navođenja količine sastojaka, sadržaja proteina i hranjive vrijednosti. Izuzeci u pogledu navođenja sadržaja proteina su proizvođači hrenovki H3, H4 i H6, te u pogledu navođenja hranjive vrijednosti hrenovki H3 i H4. Rezultati su pokazali da pileće hrenovke sadrže 55 - 76% MOM-a sa ili bez dodatka čistog pilećeg mesa (10 - 15%) i pilećih kožica. Analiza varijanse pokazala je postojanje značajnih razlika ($p<0,05$) u težini, sadržaju vode i aw-vrijednosti između uzoraka pilećih hrenovki različitih proizvođača prije i poslije kuhanja. Kuhanje je značajno uticalo na sadržaj vode i aw-vrijednost kod većine uzoraka, ali ne na težinu pilećih hrenovki. Sadržaj vode svježih hrenovki iznosio je 52,87% - 61,06%, a kuhanih 50,38% - 62,42%. Aw-vrijednost pilećih hrenovki prije kuhanja bila je 0,941 - 0,964, a poslije kuhanja 0,941 - 0,958. Težina hrenovki prije kuhanja se kretala od 102 g do 195,5 g, a poslije kuhanja od 98 g do 204,5 g. Sa povećanjem sadržaja vode nakon kuhanja povećavala se i aw-vrijednost pilećih hrenovki.

Ključne riječi: *pileće hrenovke, deklaracija, težina, sadržaj vode, aw-vrijednost*

Summary

In this study, the declaration, weight, water content and aw-value of chicken hot dogs from six different domestic manufacturers were analysed.
Domestic chicken hot dog manufacturers do not meet the declaration requirements in ingredients quantity, protein content and nutritional value declarations. Exceptions to protein content declaration are H3, H4 and H6 manufacturers, as well as H3 and H4 in the declaration of nutritional value. The results showed that chicken hot dogs contain 55 - 76% mechanically deboned meat with or without the addition of lean chicken meat (10 - 15%) and chicken skin. Analysis of variance (ANOVA) showed

[1] Poljoprivredno-prehrambeni fakultet Univerziteta u Sarajevu / *Faculty of Agriculture and Food Sciences University of Sarajevo*

significant differences ($p<0.05$) in weight, water content and aw-values between samples of chicken hot dogs from different manufacturers before and after cooking. Cooking has a significant impact on the water content and the aw-value in most samples, but not on the weight of chicken hot dogs. The water content of fresh chicken hot dogs was between 52.87% and 61.06% and cooked between 50.38% and 62.42%. Aw-value of chicken hot dogs before cooking was from 0.941 to 0.964 and after cooking from 0.941 to 0.958. Weight of hot dogs before cooking ranged from 102 g to 195.5 g and after cooking from 98 g to 204.5 g. With the increase of water content after cooking, the aw-value of chicken hot dogs also increased.

Key words: *chicken hot dogs, declaration, weight, water content, aw-value*

UVOD

Tokom zadnjih pet godina Agencija za sigurnost hrane u Bosni i Hercegovini donijela je nekoliko novih pravilnika vezanih za meso i mesne proizvode, mikrobiološku ispravnost, upotrebu dodatnih sastojaka (aditivi, začini, boje i slično), deklariranje proizvoda i drugo. Nova pravila iziskuju od proizvođača mesnih proizvoda dodatni napor u ispunjavanju određenih uslova za mesne proizvode, a sve u cilju dobre sigurnosti i kvaliteta mesnih proizvoda ponuđenih na tržistu Bosne i Hercegovine. Prema Pravilniku o usitnjenom mesu, poluproizvodima i proizvodima od mesa (Sl. Gl. BiH, 82/13) (Anonimus, 2013a) fino usitnjene barene kobasice proizvode se i stavljaju u promet kao "hrenovka", "pariška kobasica", "ekstra kobasica" ili "posebna" i "bijela kobasica", a mogu se proizvoditi i druge vrste srodnih proizvoda. Najpoznatiji predstavnici ove vrste kobasica su hrenovke. Hrenovke imaju specifičnu recepturu, a karakteristična aroma i boja dobijaju se tokom toplotne obrade koja uključuje kuhanje i dimljenje. Prema naprijed navedenom pravilniku hrenovka je proizvod od različitih vrsta, svinjskog i/ili goveđeg mesa, mesa peradi, masnog i vezivnog tkiva i dodatnih sastojaka. Nadjev za hrenovku puni se u prirodne ili u vještačke omotače odgovarajućeg promjera. U pravilniku o pružanju informacija potrošačima o hrani (Sl.glasnik BiH, 68/2013) (Anonimus, 2013b) navodi se koje obavezne informacije o hrani trebaju biti navedene na deklaraciji. Tako je obavezno navođenje sljedećih podataka: naziv hrane, spisak sastojaka, sastojci koji mogu izazvati alergije, količina sastojaka, neto količina hrane, datum minimalnog trajanja ili datum upotrebe („upotrebljivo do“), svi posebni uslovi skladištenja ili/i uslovi upotrebe, naziv i adresa subjekta u poslovanju s hranom, država porijekla ili mjesto porijekla, uputstvo za upotrebu, ako se hrane ne bi mogla upotrijebiti bez tog uputstva i deklaraciju hranjive vrijednosti (energetska vrijednost, količina masti, zasićenih masnih kiselina, ugljikohidrata, šećera, proteina i soli). Osim naprijed navedenog, prema Pravilniku o usitnjenom mesu, poluproizvodima i proizvodima od mesa (Sl. glasnik 82/2013) (Anonimus, 2013a) deklaracija mora sadržavati podatke o najmanjem sadržaju proteina mesa u gotovom proizvodu, tj. ukupnih proteina mesa. Prema istom

pravilniku hrenovke ne smiju sadržavati manje od 10% proteina mesa, a relativan sadržaj proteina vezivnog tkiva u proteinima mesa ne smije biti veći od 10%.
Hrenovke spadaju u grupu emulgovanih proizvoda. Proizvodnja pilećih hrenovki tokom vremena raste. Ona je zasnovana na upotrebi pilećeg mesa kao osnovnog izvora sirovine i pilećih kožica kao izvora masti. No, međutim, tokom vremena u pileće hrenovke sve više se dodaje mehanički otkošteno meso (MOM). Nažalost, danas se u našoj zemlji najviše proizvode jeftine hrenovke čija je osnovna sirovina mehanički otkošteno mesdo (MOM) bez dodatka čistog mesa. Smatra se da se dobar kvalitet pilećih hrenovki može postići ako se pored MOM-a, doda i mala količina čistog pilećeg mesa (10 - 15%). S naučne strane, pored dobijanja boljeg kvaliteta obezbijeđuje se i dovoljna količina proteina radi zadovoljavanja zakonskih propisa a također i dovoljna količina proteina koji će vezati dodatu vodu. Voda i mast se dodaju iz nekoliko razloga: dobijanje dobre emullize, poboljšanje senzornog kvaliteta (teksture, sočnosti, žvakljivosti) te povećanja prinosa gotovog proizvoda (Operta, 2015). Količina dodate vode kreće se od 10 do 30%. Sadržaj vode u gotovim hrenovkama koje navode mnogi autori (Franjčec i sar., 2011; Pleadin i sar., 2009; Price i sar., 2009; Vomberger i sar., 1987; Vukić, 2005) kreće se u rasponu 52,92% - 63,25%. Babić (2006) u svojim istraživanjima navodi nešto veći prosječan sadržaj vode (65,43%) za pileće hrenovke. Zbog visokog sadržaja vode hrenovke su dosta nestabilne, imaju kratak rok trajanja od 30 do 45 dana i moraju se čuvati na niskim temperaturama (do 4 °C). Aw-vrijednost je parametar koji je važan za stabilnost mesnih i drugih prehrambenih proizvoda. Prema navodima Heinz i Hautzinger (2007) aw-vrijednost toplotno obrađenih kobasica (gdje spadaju i hrenovke) kreće se od 0,960 - 0,980, što ih prema kriterijima Dibirasulaev i sar. (2005) svrstava u lako kvarljive proizvode, koji se moraju čuvati na niskim temperaturama do 4 °C. U Bosni i Hercegovini proizvede se značajna količina pilećih hrenovki, koje nisu dovoljno istražene u pogledu kvaliteta i deklarisanja. U ovom istraživanju koristit će se uzorci pilećih hrenovki od šest najvećih domaćih proizvođača hrenovki koji svoje proizvode nude u supermarketima na području Sarajevskog kantona.
Cilj istraživanja je analizirati deklaraciju, te ispitati da li toplotna obrada pred konzumaciju značajno utiče na težinu, sadržaj vode i aw-vrijednost pilećih hrenovki.
Hipoteza rada: Deklaracije pilećih hrenovki ne ispunjavaju uslove važećih pravilnika. Postoji značajan uticaj toplotne obrade pred konzumaciju na težinu, sadržaj vode i aw-vrijednost pilećih hrenovki.

MATERIJAL I METODE RADA

Za potrebe istraživanja kupljeno je šest uzoraka pilećih hrenovki u tri ponavljanja (tri pakovanja po četiri komada hrenovki po uzorku) proizvedenih od različitih domaćih proizvođača mesnih proizvoda. Uzorci su šifrirani sljedećim šiframa: H1, H2, H3, H4, H5 i H6. Nakon kupovine, hrenovke su čuvane na temperaturi do 4 °C do početka laboratorijskih analiza na Poljoprivredno-prehrambenom fakultetu Univerziteta u

Sarajevu. Pileće hrenovke podvrgle su se toplotnoj obradi na osnovu preporuka na ambalaži, tj. hrenovke su se potopile u hladnu vodu, koja se potom zagrijala do vrenja (ključanja) što je u prosjeku trajalo 3 - 4 minute. Težina prije i poslije toplotne obrade hrenovki (pakovanje sa četiri komada) mjerena je pomoću vage sa tačnošću ±0,05g. Na osnovu težine hrenovki prije i nakon kuhanja izračunat je gubitak mase (kalo) tokom kuhanja izražen u postotcima. Određivanje sadržaja vode rađeno je po BAS ISO (1442:2007) metodi. Aw-vrijednost mjerena je pomoću Aw-metra (LabSwift-aw, Novasina, Švajcarska). Dobijeni podaci obrađeni su u SPSS 16.0, kroz analizu varijanse. Kada je uticaj bio značajan razlike između uzoraka su analizirane pomoću Tukey testom. Za ispitivanje uticaja kuhanja na težinu, sadržaj vode i aw-vrijednost korišten je T-test. Za dokazivanje postojanja korelacije između pojedinih svojstava, korišten je Pearsonovog koeficijent korelacije.

REZULTATI RADA I DISKUSIJA

U tabeli 1. predstavljeni su podaci uzeti s deklaracija pilećih hrenovki različitih domaćih proizvođača. Svi proizvođači pilećih hrenovki ispunjavali su uslove Pravilnika o pružanju informacija potrošačima o hrani (Sl.glasnik BiH, 68/2013) (Anonimus, 2013b) u pogledu obaveznog navođenja podataka (naziv hrane, naziv i adresa subjekta u poslovanju s hranom, država porijekla ili mjesto porijekla, neto masa hrane, datum minimalnog trajanja ili datum upotrebe „upotrebljivo do“, svi posebni uslovi skladištenja ili/i uslovi upotrebe, uputstvo za upotrebu, ako se hrane ne bi mogla upotrijebiti bez tog uputstva, spisak sastojaka), ali ne i količine pojedinih sastojaka. Proizvođači u proizvodnji hrenovki koriste 55% – 76% mehanički otkoštenog mesa. Za hrenovke H2 i H5 navedeno je da se koristi mehanički otkošteno meso (MOM), ali je njegova količina nepoznata. Samo dva proizvođača navode da koriste i dio čistog mesa u količini od 10% (H1) i 15% (H6). Količina pilećih kožica navedena je samo na deklaraciji hrenovki H6 (5%). Jedan proizvođač ne koristi, dok su četiri ostala proizvođača navela da u recepturi koriste pileću kožicu, ali se ne zna njena količina. Ako kao parametar kvaliteta sirovine, uzmemo odnos postotka MOM-a i čistog pilećeg mesa, po tome, najbolji kvalitet imaju hrenovke H6 sa 55% MOM-a i 15% čistog pilećeg mesa. Kada su u pitanju aditivi, navođenje aditiva dosta varira. Na nekim deklaracijama navedeni su nazivi grupa aditiva kao što su stabilizatori, emulgatori, antioksidanti i slično. Opet, na drugim deklaracijama naveden je naziv aditiva (kao naprimjer natrijev difosfat, natrij nitrit) ili E-broj (E450, E621, E250 itd.). No, važno je napomenuti da bez obzira na različito navođenje, svi proizvođači su naveli da u hrenovke dodaju stabilizatore/emulgatore, antioksidante i konzervanse. Ostali sastojci su uglavnom isti za većinu uzoraka hrenovki, s izuzetkom da se kukuruzni skrob dodaje samo u recepturu pilećih hrenovki H3.
Prema Pravilniku o usitnjenom mesu, poluproizvodima i proizvodima od mesa (Sl. Gl. BiH, 82/13) (Anonimus, 2013a), obaveza proizvođača je da na deklaraciji navedu količinu ukupnih proteina. Tri proizvođača (H3, H4 i H6) ispunjavaju uslove ovoga pravilnika, jer su naveli količinu ukupnih proteina, a ona je veća od 10% što je

Tabela 1. Podaci sa deklaracije pilećih hrenovki različitih proizvođača
Table 1. Labelling and composition of chicken hot dogs from different manufacturers

Pileće hrenovke/ *Chicken hot dogs*	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**
Naziv hrane/*Name of the food*	+	+	+	+	+	+
Naziv i adresa proizvođača/ *Manufakcurer's name and adrress*	+	+	+	+	+	+
Država porijakla/*Country of origin*	+	+	+	+	+	+
Neto masa (g)/*Total Weight (g)*	500	230	200	100	200	200
Temperatura čuvanja/ *Storage Temperature*	0-4 ºC	0-4 ºC	0-4 ºC	0-4 ºC	0-4 ºC	0-4 ºC
Upotrebljivo do: / *Expiration date:*	+	+	+	+	+	+
Uputstvo za upotrebu/*Manual*	+	+	+	+	+	+
Mehanički otkošteno meso/ *Mechanically Deboned Meat*	60%	+	70%	76%	+	55%
Čisto pileće meso/ *Lean poultry meat*	10 %	-	-	-	-	15 %
Pileće kožice/*Chicken skin*	-	+	+	+	+	5%
Voda/*Water*	+	+	+	+	+	+
Kuhinjska so/*Salt*	+	+	+	+	+	+
Sojini proteini/*Soy proteins*	+	+	+	+	+	+
Kukuruzni skrob/*Corn starch*	-	-	+	-	-	-
Šećer/*Sugar*	+	+	+	Dekstroza	Dekstroza	-
Stabilizatori i emulgatori/ *Stabilisers and Emulsifiers*	E450; E451; E452	E450; E451	E450; E451; E516	Natrijev difosfat	E450	E450
Zgušnjivači/*Hydrocolloid gums*	-	-	E401	-	-	E407; E412
Antioksidanti/*Antioxidants*	E300	E801	E301	Na-askorbat; Na-laktat	E330; E316	E300
Pojačivač okusa/*Flavour Enhancer*	E621	E621	-	-	+	E621
Konzervansi/ *Preservatives*	E250	E250	E250; E262	Natrijev nitrit	E262	E250
Začini/*Spices*	+	+	-	+	+	+
Aroma/*Aroma*	+	-	+		-	-
Energetska vrijednost/*Energy value*	-	-	211 kcal	236 kcal	-	-
Količina masti/*Amount of fat*	-	-	18,2 g	20 g	-	-
Zasićene masne kiseline/ *Saturated fatty acids*	-	-	5,4 g	6 g	-	-
Ugljikohidrati/ *Carbohydrates*	-	-	1,8 g	1 g	-	-
Šećeri/*Sugar*	-	-	0,01 g	0,5 g	-	-
Proteini/*Proteins*	-	-	10 g	13 g	-	11 g
So/*Salt*	-	-	1,4 g	1,7 g	-	-
Vlakna graška/ *Peanut fiber*	+	-	-	0,5 g	-	

* + Navedeno na deklaraciji

minimalan sadržaj proteina po pravilniku. Ostala tri proizvođača nisu ispunjavala uslove u pogledu navođenja sadržaja ukupnih proteina.
Prema Pravilniku o pružanju informacija potrošačima o hrani (Sl. glasnik BiH, 68/2013) (Anonimus, 2013b) proizvođači se obavezuju da na deklaraciji moraju navesti podatke o hranjivoj vrijednosti proizvoda. Uslove iz ovoga pravilnika ispunili su proizvođači hrenovki H3 i H4 koji su naveli podatke o hranjivoj vrijednosti, dok ostali nisu. Preporučena temperatura čuvanja (do 4 °C) istaknuta je na svim deklaracijama. S obzirom da su hrenovke proizvod koji se priprema na osnovu proizvođačke specifikacije, razumljivo je da specifikacije različitih proizvođača hrenovki nisu identične (Prica i sar., 2009). Zato su jasne razlike od proizvođača do proizvođača u kvalitetu upotrijebljene sirovine i dodataka.

U tabeli 2. predstavljeni su podaci o težini hrenovki prije i poslije kuhanja i gubicima tokom kuhanja. Težina hrenovki prije kuhanja kretala se u rasponu od 102 g do 195,5 g, a poslije kuhanja od 98 g do 204,5 g. Analiza varijanse pokazala je postojanje značajnih razlika ($p<0,05$) u težini između različitih uzoraka hrenovki prije i poslije kuhanja. Razlike su bile značajne između svih uzoraka hrenovki, osim između hrenovki H1 i H6. Iako je tokom kuhanja hrenovki dolazilo do povećanja ili smanjenja težine pojedinačno za svaki uzorak, te promjene nisu bile značajne ($p>0,05$). Najveću težinu prije i poslije kuhanja imaju hrenovke H3, a najmanju hrenovke H4. Hrenovke H1 i H3 su kuhanjem dobile dok su ostale hrenovke izgubile na težini. Najveća promjena uočena je kod hrenovki H3, čiji prirast u masi je iznosio čak 9 grama, te kod hrenovki H5 koje su kuhanjem prosječno izgubile 6 grama. Težina hrenovki H2 se najmanje promijenila, odnosno, kuhanjem su izgubile tek 0,5 grama. Razlog zašto su hrenovke H3 povećale svoju težinu tokom kuhanja, tj. upile vodu, može biti zbog toga što se iz deklaracije vidi da se jedino u ove hrenovke dodaje kukuruzni skrob. Uloga dodatog skroba između ostalog je vezivanje vode. Razlozi većeg gubitka vode tokom kuhanja kao što je slučaj kod hrenovki H5, mogu biti da je dodato previše vode u recepturu uz nedovoljan sadržaj proteina i stabilizatora koji mogu pospješiti to vezivanje, visoka temperatura pri kuterovanju, nedosljednost kod dodavanja dodataka i slično.

Tabela 2. Težina pilećih hrenovki (g) (srednja vrijednost ± standardna devijacija)
Table 2. Weight of chicken hot dogs (g) (mean ± SD)

Hrenovke (*Hot Dogs*)	Težina prije kuhanja (*Weight before cooking*)	Težina poslije kuhanja (*Weight after cooking*)	Gubitak kuhanjem (*Loss by cooking*)	T-test (p<0,05)	
H1	117,5^{d} ± 0,71	119,0^{d} ± 0,00	+1,5	0,095	NS
H2	154,5^{c} ± 2,12	154,0^{c} ± 2,83	-0,5	0,860	NS
H3	195,5^{a} ± 0,71	204,5^{a} ± 4,95	+9	0,126	NS
H4	102,0^{e} ± 2,82	98,0^{e} ± 2,83	-4	0,293	NS
H5	171,0^{b} ± 1,41	165,0^{b} ± 1,41	-6	0,051	NS
H6	111,5^{d} ± 0,71	110,5^{d} ± 0,71	-1	0,293	NS

Srednje vrijednosti unutar istog stupca s različitim slovima (a-e) se značajno razlikuju (p<0,05)/ *Means within same column with different letters (a-e) are significantly different (p<0,05)/* NS (p>0,05) – nije značajno (*not significant*); * (p<0,05) – značajno (*significant*)

U tabeli 3. prikazan je prosječan sadržaj vode u pilećim hrenovkama prije i poslije kuhanja. Rezultati ispitivanja pokazali su da su varijacije u sadržaju vode prije i poslije kuhanja bile značajne između većine uzoraka hrenovki različitih proizvođača. Sadržaj vode hrenovki se značajno povećavao (H1 i H3) ili smanjio (H2, H5 i H6) tokom kuhanja izuzev kod uzorka hrenovke H4 gdje gubitak od 2,08% nije bio značajan. Sadržaj vode u ispitivanih hrenovkama u svježem (52,87% - 61,06%) i kuhanom stanju (50,38% - 62,42%), kretao se u rasponu (52,92% - 63,25%) koji navode mnogi autori (Franjčec i sar., 2011; Pleadin i sar., 2009; Price i sar., 2009; Vomberger i sar., 1987; Vukić, 2005). Nešto veći prosječni sadržaj vode u pilećim hrenovkama (65,43%) navodi Babić (2006) u svojim istraživanjima.

Tabela 3. Sadržaj vode u pilećim hrenovkama (%) (srednja vrijednost ± standardna devijacija)
Table 3. Water content of chicken hot dogs (%) (mean ± SD)

Hrenovke (*Hot Dogs*)	Sadržaj vode prije kuhanja (*Water content before cooking*)	Sadržaj vode poslije kuhanja (*Water content after cooking*)	Razlika (*Difference*)	T-test (p<0,05)	
H1	61,06^{a} ± 0,85	62,42^{a} ± 0,77	+1,36	0,004	*
H2	54,95^{c} ± 0,78	54,50^{c} ± 0,09	-0,45	0,034	*
H3	58,11^{b} ± 0,12	59,93^{b} ± 0,08	+1,82	0,003	*
H4	52,87^{d} ± 0,44	50,79^{d} ± 0,75	-2,08	0,077	NS
H5	53,95^{cd} ± 0,60	50,38^{d} ± 0,77	-3,57	0,035	*
H6	58,64^{b} ± 0,08	58,19^{b} ± 0,12	-0,45	0,049	*

Srednje vrijednosti unutar istog stupca s različitim slovima (a-e) se značajno razlikuju (p<0,05)/ *Means within same column with different letters (a-e) are significantly different (p<0,05)/* NS (p>0,05) – nije značajno (*not significant*); * (p<0,05) – značajno (*significant*)

Analiza varijanse pokazala je da postoje statistički značajne ($p<0,05$) razlike u aw-vrijednosti između uzoraka pilećih hrenovki prije i poslije kuhanja (tabela 4). Značajno veću aw-vrijednost prije kuhanja imale su hrenovke H5 u odnosu na hrenovke H3 i H4. Poslije kuhanja hrenovke H4 su imale značajno manju aw-vrijednost u odnosu na hrenovke H1, H3 i H6. Uticaj kuhanja bio je značajan na aw-vrijednost hrenovki H3 i H4, ali sa suprotnim efektom. Dok je kod hrenovki H3 došlo do značajnog povećanja aw-vrijednosti, kod hrenovki H4 aw-vrijednost tokom kuhanja se značajno smanjila. Ukupno gledajući aw-vrijednost svježih (0,941 - 0,964) i kuhanih (0,941 - 0,958) hrenovki bila je dosta slična, te je bila nešto niža od raspona aw-vrijednosti koji za ovu vrstu kobasica (0,960 - 0,980) navode Heinz i Hautzinger (2007). Prema kriterijima Dibirasulaev i sar. (2005) sve hrenovke iz ovoga ispitivanja se mogu svrstati u lahko kvarljive proizvode, te se moraju čuvati na niskim temperaturama do 4 °C. Između sadržaja vode i aw-vrijednosti pilećih hrenovki poslije kuhanja postojale je veoma jaka korelaciona veza ($r = 0,78^{**}$), što znači da što je bio veći sadržaj vode hrenovki poslije kuhanja, bila je veća i aw-vrijednost.

Tabela 4. Aw vrijednost pilećih hrenovki (srednja vrijednost ± standardna devijacija)
Table 4. Aw-value of chicken hot dogs (mean ± SD)

Hrenovke (*Hot Dogs*)	Aw vrijednost prije kuhanja (*Aw-value before cooking*)	Aw vrijednost poslije kuhanja (*Aw-value after cooking*)	Razlika (*Difference*)	T-test (p<0,05)	
H1	$0,952^{abc} \pm 0,002$	$0,955^{a} \pm 0,004$	+0,003	0,412	NS
H2	$0,954^{abc} \pm 0,005$	$0,952^{ab} \pm 0,004$	-0,002	0,688	NS
H3	$0,941^{c} \pm 0,002$	$0,958^{a} \pm 0,004$	+0,017	0,028	*
H4	$0,950^{bc} \pm 0,001$	$0,941^{b} \pm 0,000$	-0,009	0,012	*
H5	$0,964^{a} \pm 0,006$	$0,949^{ab} \pm 0,007$	-0,015	0,061	NS
H6	$0,956^{ab} \pm 0,001$	$0,954^{a} \pm 0,002$	-0,002	0,300	NS

Srednje vrijednosti unutar istog stupca s različitim slovima (a-e) se značajno razlikuju (p<0,05)/ *Means within same column with different letters (a-e) are significantly different (p<0,05)*/ NS (p>0,05) – nije značajno (*not significant*); * (p<0,05) – značajno (*significant*)

ZAKLJUČAK

Proizvođači pilećih hrenovki ispunjavaju uslove pravilnika o deklarisanju u pogledu navođenja svih osnovnih podataka na deklaraciji, izuzev količine pojedinih sastojaka. Hrenovke proizvođača H1 i H6 se na osnovu sastava osnovne sirovine mogu smatrati kvalitetnijim u odnosu na ostale koje sadrže samo mehanički otkošteno meso bez dodatka čistog mesa. Hrenovke proizvođača H3, H4 i H6 ispunjavaju uslove u pogledu navođenja i sadržaja ukupnih proteina na deklaraciji. Podaci o hranjivoj vrijednosti navedeni su samo kod hrenovki proizvođača H3 i H4, čime oni ispunjavaju uslove pravilnika o deklarisanju hranjive vrijednosti. Hipoteza u pogledu ispunjavanja uslova iz pravilnika je djelimično potvrđena. Pileće hrenovke različitih domaćih proizvođača značajno su se razlikovale u težini, sadržaju vode i aw-vrijednosti. Kuhanje je imalo značajan uticaj na sadržaj vode i aw-vrijednost, ali ne na težinu pilećih hrenovki. Sa povećanjem sadržaja vode nakon kuhanja povećava se i aw-vrijednost pilećih hrenovki. Hipoteza u pogledu uticaja kuhanja na pojedine parametre je potvrđena kod sadržaja vode i aw-vrijednosti, ali ne i kod težine pilećih hrenovki.

LITERATURA

Anonimus. (2008). Pravilnik o upotrebi prehrambenih aditiva osim boja i zaslađivača u hrani. Službeni glasnik BiH, broj 83.

Anonimus. (2013a). Pravilnik o usitnjenom mesu, poluproizvodima i proizvodima od mesa. Službeni glasnik BiH, broj 82.

Anonimus. (2013b). Pravilnik o pružanju informacija potrošačima o hrani. Službeni glasnik BiH, broj 68.

Babić, S. (2006). Utjecaj sirovine na kvalitet barenih kobasica uključujući HACCP kontrolu. Magistarski rad. Poljoprivredno-prehrambeni fakultet Univerziteta u Sarajevu.

Dibirasulaev, M. A., Uskokov, M. H., Fatjanov, E. V. (2005). Pravilno li mi hranim kolbasu? Holodiljnijbiznes, broj 2.C., str. 38-39.

Franjčec, I., Njari, B., Cvrtila-Fleck, Ž. (2011). Ocjena tržišne kakvoće obarenih kobasica. Meso. Vol. XIII, broj 5, str. 351-353.

Heinz, G., Hautzinger, P. (2007). Meat processing tehnology. For small-to-medium-scale producers, FAO.

Operta, S. (2015). Tehnologija proizvoda od usitnjenog mesa. Skripta-nastavni materijal. Poljoprivredno-prehrambeni fakultet Univerziteta u Sarajevu.

Pleadin, J., Perši, N., Vulić, A., Đugum, J. (2009). Kakvoća trajnih, polutrajnih i obarenih kobasica na hrvatskom tržištu. Hrvatski časopis za prehrambenu tehnologiju, biotehnologiju i nutricionizam, broj 4 (3-4), str. 104-108.

Prica, N., Baltić, M., Teodorović, V., Petrović, J., Rackov, O. (2009). Uporedna analiza hemijskih parametara kvaliteta viršli. Arhiv veterinarske medicine, vol. 2, broj 1, str. 29-37.

Vomberger, B., Čerin, T., Jurković, B. (1987). Promene hemijskog sastava i organoleptičkih karakteristika hrenovki pri višednevnom držanju u skladištu, odnosno prometu. Tehnologija mesa, broj 5, str. 141-144.

Vukić, D. (2005). Hemijski parametri kvaliteta kobasica namenjenih ishrani vojske Srbije i Crne Gore. Specijalistički rad.Fakultet veterinarske medicine u Beogradu.

IMPACT OF SOURDOUGH ADDITION ON THE BREAD QUALITY*

UTICAJ DODATKA KISELOG TIJESTA NA KVALITET HLJEBA

Vesna Rašević[1], Amila Vranac[2], Sanja Oručević Žuljević[2]

Original scientific paper

Summary

The aim of undertaken research was to examine the impact of sourdough addition on the quality parameters of fresh breads, and the same bread samples during the five days of storage as well. For that purpose, breads were made from T-500, rye and wholemeal wheat flour, without and with added sourdoughs in the form of starter culture and preferment. The analysis of fresh samples included examination of physical (weight, friability, height, cross-section area, texture – firmness), chemical (water content, pH value, acidity level) and sensory (taste and solubility of crust and crumb, aroma, scent, freshness and overall impression) properties. During the breads storage, water content, texture and sensory attributes were monitored. The obtained results showed that sourdough addition had positive impact on the quality of breads. Physical parameters of breads, mainly texture, were improved as well as sensory properities, especially aroma. The positive impact of sourdough addition was notably evident for breads made from rye and wholemeal wheat flour. With prolonged time of breads storage, water content, texture – firmness values and grades for sensory attributes had negative trend, which was less drastic for breads produced with addition of sourdoughs. Thus, using the sourdough with the aim of nutritional, technological and sensory quality improvement of breads can be recommended.

Key words: *Bread, sourdough, quality parameters, storage*

Rezime

Cilj istraživanja je bio ispitati uticaj dodatka kiselog tijesta na parametre kvaliteta svježih hljebova, te istih uzoraka hljeba tokom pet dana skladištenja. Za tu namjenu su proizvedeni hljebovi od pšeničnog T-500, raževog i integralnog pšeničnog brašna, bez i sa dodatkom kiselog tijesta u formi starter kulture i predtijesta. Analize svježih hljebova su uključivale ispitivanje fizičkih (težina, mrvljivost, visina, površina poprečnog presjeka, tekstura – čvrstoća), hemijskih (sadržaj vlage, pH vrijednost, stepen kiselosti) i senzornih (okus i topivost kore i sredine, aroma, miris, svježina i

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Bakery “Croissant”, Foča, Bosnia and Herzegovina
[2] Faculty of Agriculture and Food Sciences, University of Sarajevo, Bosnia and Herzegovina

ukupna dopadljivost) svojstava. Tokom perioda skladištenja hljebova, praćene su promjene u sadržaju vlage, teksturi i senzornim svojstvima hljebova. Dobijeni rezultati su pokazali da je dodatak kiselog tijesta imao pozitivan uticaj na kvalitet hljebova. Tako su unaprijeđeni fizički parametri hljebova, naročito tekstura, kao i senzorna svojstva, a posebno aroma. Pozitivan uticaj dodatka kiselog tijesta je bio naročito evidentan kod hljebova proizvedenih od raževog i integralnog pšeničnog brašna. Sa produženjem perioda skladištenja hljebova, vrijednosti sadržaja vlage, teksture-čvrstoće i senzorne ocjene su imale negativan trend, a koji je bio manje drastičan za hljebove proizvedene sa dodatkom kiselih tijesta. Stoga, mogu se dati preporuke za korištenje kiselih tijesta, sa ciljem nutritivnog, tehnološkog i senzornog unapređenja hljebova.

Ključne riječi: *Hljeb, kiselo tijesto, parametri kvaliteta,, skladištenje*

INTRODUCTION

Although it is well known since ancient times (Poutanen *et al*., 2009), nowadays there is an increasing trend in sourdough utilization during the breads production, due to the consumers' demans for healthy and functional foods (Thiele *et al.*, 2002; Lopez *et al.*, 2003). Traditionally sourdough (preferment) is made by spontaneous fermentation of flour, water and salt mixture, in presence of autochtonous lactic acid bacteria and yeasts (Hammes & Gänzle, 1998, Chavan & Chavan, 2011). However, recent production of sourdough is carried out by the addition of starter cultures, which provides better control of fermentation process. There are many advantages of sourdough addition during the bread production, such as aroma, rheological properties and storage stability improvement (Katina *et al*., 2006a, Katina *et al*., 2006b., Arendt *et al*., 2007, Plessas *et al*., 2007). The aim of undertaken research was to examine the impact of sourdough (as preferment and starter culture) addition on the quality characteristics of fresh made breads, and the same samples of bread during the totally five days of storage as well.

MATERIAL AND METHODS

As a main material for breads production, three types of flour were used: wheat (T-500), rye (T-1250) and wholemeal wheat flour. Along with flours, tap water, yeast, salt, shortening, starter culture emulsion and preferment were used as well. In dependence of used flour and addition of starter culture or preferment, three types of breads were produced, whereals control breads were made according to straight-dough recipe without the addition of any acidifying material (tab. 1).
Production of breads was carried out in bakery „Croissant“ (Foča, B&H) throught the following steps: preparation, weighing and mixing of ingredients (5 min. fast, 2 min. slow), dough resting (10 min., 27-28 °C), cutting dough into pieces (420 g), rounding and resting (30 min), final shaping and moulding, fermentation (36 °C, 70-75% RH,

60 min.), baking (220 °C, 20-22 min) and steaming at the beginning, breads cooling and moulds removal. Each bread sample was produced in five replicates and the total number of samples was 45.

Table 1. Bread samples

Flour	*Dough type*		
	Control	Starter culture	Preferment
T-500	W	W-SC	W-SD
Rye T-1250 + wholemeal wheat + T-500	R	R-SC	R-SD
Wholemeal wheat + T-500	IW	IW-SC	IW-SD

The quality analysis of fresh produced breads included examination of physical, chemical and sensory properties. Physical parameters analysis included determination of: weight (g), friability (g), height (cm), cross-section area in cm^2 (Koizumi placom digital planimeter, Japan) according to Oručević (2016), texture – firmness as compression force in N (TA.XT *plus* Texture Analyser, Stable Micro Systems, UK). Among chemical properties, water contet (%), pH value and acidity level were analyzed according to Kaluđerski & Filipović (1998). All the analysis were done as duplicates. Sensory evaluation of breads was carried out by five trained panelists. The following sensory attributes of breads were evaluated: taste and solubility of crumbs and crusts, aroma, scent, freshness and overall impression. Sensory parameters were graded on a scale 1-5 (1 – the lowest, 5 – the highest score), according to quantitative descriptive method. During the following days of breads storage, changes in water content, texture-firmness and sensory atributtes were investigated daily.

Statistical analysis

Statistical analysis included descriptive and two-factorial analysis of variance (ANOVA). Analysis of variance (MS Excel 2013), followed by *post hoc* Tukey's test (0.05), examined the impact of flour and dough type on evaluated physical-chemical and sensory parameters.

RESULTS AND DISCUSSION

Results of physical-chemical analysis (tab. 2) demonstrate notable differences between different types of bread samples. Moreover, analysis of variance revealed significant impact of flour and dough type on all evaluated parameters, whereas control samples significantly differed from those made with the addition of starter culture and preferment. Breads made from rye and wholemeal wheat flour had higher weight in comparison with T-500 breads, as expected, due to the increased presence of pentosans and other fibers which have higher water absorption and retention capacity.

Table 2. Physical-chemical parameteres of breads

		Weight (g)	Friability (g)	Height (cm)	Cross-section area (cm^2)	pH value	Acidity level
Control	W	351 ± 0.01 a	0.134 ± 0.04	7.2 ± 0 a	57.3 ± 0.5 a	5.7 ± 0.07 a	2.05 ± 0.07 a
	R	360 ± 0.01 b	0.08 ± 0.01	5.9 ± 0.07 b	47.2 ± 0.2 b	5.2 ± 0.07 b	4.0 ± 0.14 b
	IW	359 ± 0.07 b	0.105 ± 0	5.9 ± 0.07 b	51 ± 0.9 c	5.4 ± 0 a	3.2 ± 0 c
		x	x	x	x	x	x
Starter culture	W-SC	355 ± 0.01 a	0.067 ± 0.01	7.7 ± 0 a	63.4 ± 3.5 a	4.6 ± 0 a	2.55 ± 0.07 a
	R-SC	365 ± 0.07 b	0.057 ± 0.02	6.3 ± 0.07 b	51.5 ± 0.8 b	4.3 ± 0.07 b	5.5 ± 0.02 b
	IW-SC	365 ± 0 b	0.058 ± 0.005	7.0 ± 0 c	59.5 ± 0.3 c	4.3 ± 0.14 b	4.58 ± 0.1 c
		y	y	y	y	y	y
Preferment	W-SD	360 ± 0.01 a	0.098 ± 0.02	7.4 ± 0 a	61.6 ± 0.6 a	5.2 ± 0.07 a	2.75 ± 0.07 a
	R-SD	365 ± 0 b	0.069 ± 0.02	5.8 ± 0.07 b	50.9 ± 1.8 b	4.4 ± 0.07 b	5.53 ± 0.04 b
	IW-SC	365 ± 0 b	0.085 ± 0.001	6.4 ± 0.07 c	53.4 ± 0.07 c	5.3 ± 0.07 a	4.35 ± 0.07 c
		y	xy	x	y	z	y

Different letters 'a-c' in columns denote significantly different values among type of flours, while letters 'x-z' denote significantly different values among dough types (Tukey's test, $p < 0.05$).

Friability was found to be the highest in breads made without sourdough addition and in the samples made from T-500 flour. Similar results were obtained for height and cross-section area as well. As expected, the highest height and cross-section are were recorded in breads from T-500, while the lowest had rye flour based breads. Moreover, breads produced with preferement and particulary starter culture had higher height and cross-section area compared to control samples. According to Chavan & Chavan (2011), sourdough addition positively affects breads volume, which is directly correlated with height and cross-section properties. pH value of breads ranged from 4.3 (R-SC, IW-SC) to 5.7 (W) and it was in accordance to results reported by Barber *et al*. (1992) and Aplevicz *et al*. (2013). Generally, breads made with starter culture and from rye flour had the lowest pH value and the highest acidity levels.

As presented on figure 1, the water content in bread samples had negative trend during the storage. Since water evaporates from bread crumbs during the storage, the obtained results demonstrate that the moisture loss was gradual and progressive with prolonged storage. Generally, among fresh samples, rye flour based bread made with starter culture had the highest water content (47.03%), while at the end of storage period control bread with wholemeal wheat flour recorded the highest water content (24.98%).

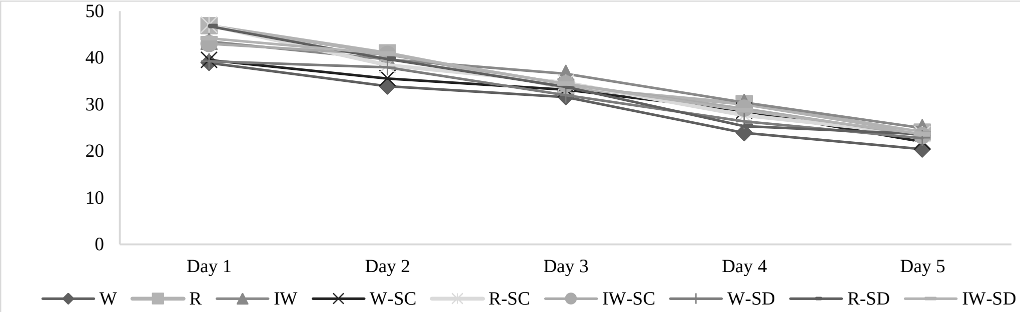


Figure 1. Changes in breads water content during the five days of storage (%)

Analysis of variance has shown that at the beginning of storage water content in breads is mostly affected by flour type, while with prolonged storage, interactions of flour and dough type influence the moisture level. However, breads made from T-500 had the lowest water content, regardless to dough type, due to the absence of fibers in flour, capable for higher water absorption and retention.

Texture-firmness of breads crumbs, measured as compression force, displayed gradual increase with prolonged storage and it ranged 0.29 (R-SC)-0.71 (IW) in fresh breads and 0.7 (W-SC)-2.19 (W) N at the end of storage period (fig. 2-A). As Gray & Bemiller (2003) reported, bread crumbs become more firm, hard and crumbly during the bread storage, due to the changes in starch fractions. These changes are mainly ascribed to gradual amylopectin retrogradation, which occurs during the five storage days. Generally, crumbs of breads made from T-500 had significantly higher firmness in comparison to those made with addition of rye and wholemeal wheat flour. Likewise, bread crumbs made with sourdoughs had lower firmness, compared to control samples. Results obtained in this study were in accordance to those reported by Symons & Brennan (2004). According to Corsetti *et al.* (1998), Crowley (2002) and Mrvičić *et al.* (2011), crumb of breads made with sourdough is charachterised by higher elasticity and lower friability. Furthermore, these type of breads remain softer and retain more moisture during the storage, due to the decreased process of amylopectin retrogradation and water migration.

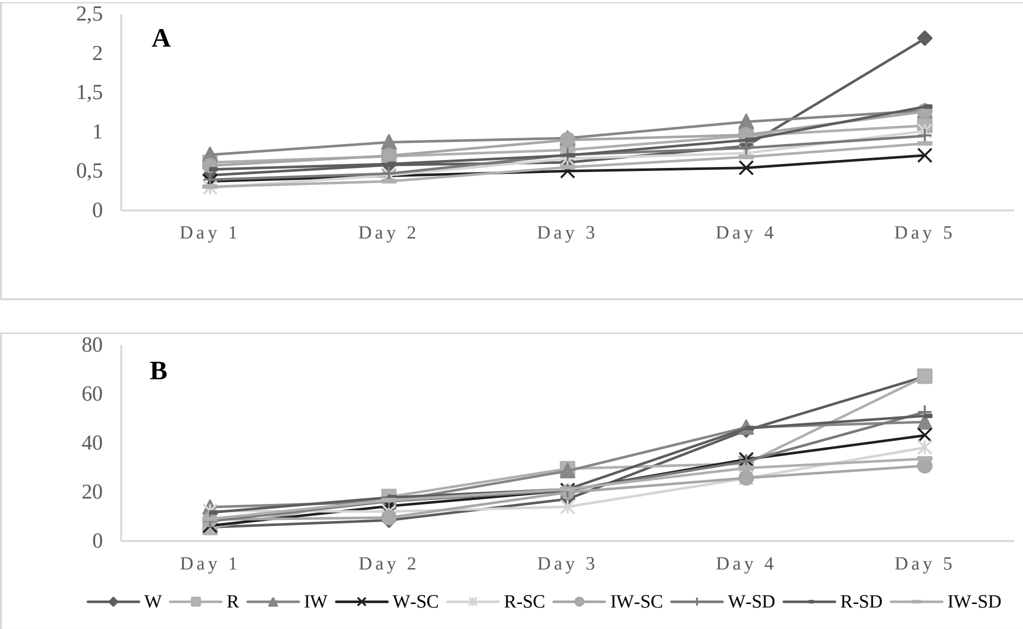


Figure 2. Changes of bread crumbs (A) and crusts (B) texture – firmness during the storage (N)

Firmness of bread crusts was higher, compared to crumbs, and varied between 5.6-13.77 N for fresh breads and 30.6-67.05 N for breads stored five days (fig. 2-B). Crusts of breads made with starter culture had the lowest firmness, while crusts of control breads had the highest by the end of storage. Clarke *et al*. (2002) and Rehman *et al*. (2007) pointed out that breads made with sourdough addition have less firm crust in comparison to control breads. Furthermore, results of study made by Banu *et al*. (2011) revealed that crusts of breads made with addition sourdough (20%) kept superior textural characteristics during the storage. This advantage could be due to the decreased migration of water from crumbs to crusts (Arendt *et al*., 2007).

Sensory evaluation revealed significant impact of dough type on observed sensory attributes of both fresh and stored breads, which was notably evident in perception of taste and solubility (fig. 3).

Generally, control samples received lower sensory grades in comparison to breads produced with acidifying materials. Moreover, the results have shown that third day of storage was crucial for making distinct differences in taste and solubility among samples, while after this days samples were quite homogeneous and hard to distinguish.

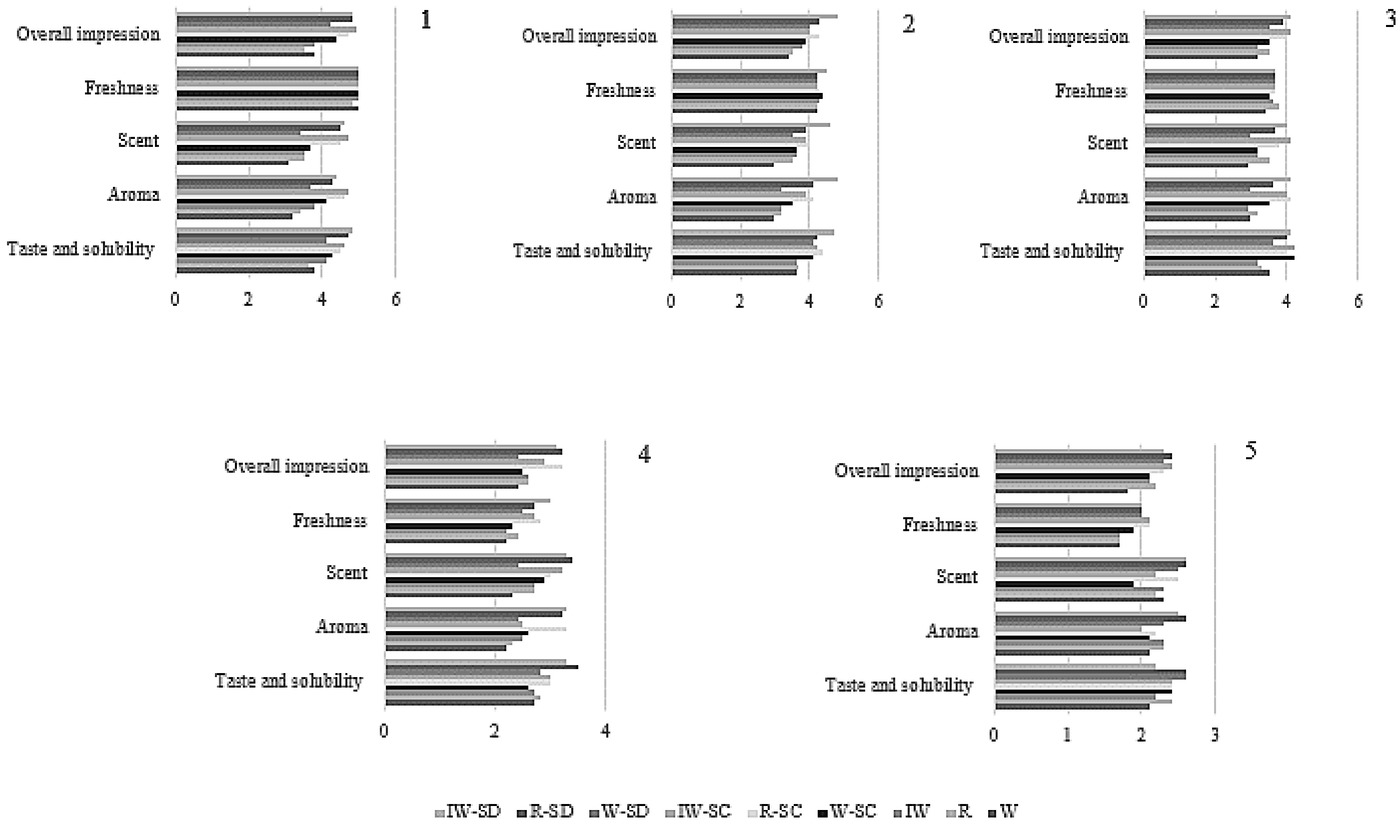


Figure 3. Average sensory grades for breads during five (1-5) days of storage (1-5 grades)

Aroma of breads was found to be affected by both dough and flour type, whereas breads made from rye and wholemeal wheat flour and with added sourdough (starter culture and preferment) had higher grades for this sensory feature. Similar results were reported by Bruemmer & Lorenz (1991), Katina *et al*. (2006a), Ganzle *et al*. (2007). According to Hansen (2002) and Chavan & Chavan (2011), the most important aromatic compounds found in sourdough are organic acids, alcohols (3-methyl-propanol, 2-phenylethanol), diacetyl and acetaldehyde.
As expected, the pleasant scent of breads (fig. 3) was apparently vanishing during their staling. The second day of storage appeared to be critical for scent perception and samples differentiation. Flour type significanlty influenced this sensory propertie during the first three days of storage, as well as dough type.
As Heenan *et al*. (2008) and Lambert *et al*. (2009) stated, freshness of bread is the most important sensory feature, highly requested by consumers. This propertie is directly dependent upon bread's water content and highly correlated with its texture. Average sensory grades given for breads freshness had negative trend with extended storage period and they were slightly higher for breads made with addition of starter culture and preferment. Generally, the highest grades for overall impression (fig. 3) of evaluated breads, during the five days of study, had samples made with sourdough addition, whereas breads with preferment were slightly better graded than those with added starter culture.

CONCLUSIONS

On the basis of conducted study, it can be concluded that addition of sourdough during breads production positively affects overall quality of final products, especially when combined with rye or wholemeal wheat flour. The positive impact was particulary evident in textural and sensory properties improvement, as well as extension of shelf life. Starter culture addition mostly influenced texture-firmness of breads, while breads made with preferment displayed slightly higher grades for all evaluated sensory attributes. Regardless of the sourdough type, recommendations for its utilization during the bread production can be given.

REFERENCES

Aplevicz, K. S., Ogliari, P. J., Sant'Anna, E. S. (2013): Influence of fermentation time on characterstics of sourdough bread. Brazilian journal of pharmaceutical sciences, 49: 233-239.

Arendt, E. K., Ryan, L. A. M., Bello, F. D. (2007): Impact of sourdough on the texture of bread. Food microbiology, 24: 165-174.

Banu, I., Vasilean, I., Aprodu, I. (2011): Quality evaluation of the sourdough rye breads. The Annals of the University Dunarea de Jos of Galati Fascicle VI – Food Technology, 35 (2): 94-105.

Barber, B., Ortola, C., Barber, S., Fernandez, F. (1992): Storage of packaged white bread. III. Effects of sour dough and addition of acids on bread characterstics. Z. Lebensm. Unters. Forsch, 194: 442-449.

Bruemmer, J. M. & Lorenz, K. (1991): European development in wheat sourdoughs. Cereal food world, 36: 310-312.

Chavan, R. S. & Chavan, S. R. (2011): Sourdough technology – a traditional way for wholesome foods: A review. Comprehensive reviews in food science and food safety, 10: 170-183.

Clarke, C., Schober, T. J., Arendt, E. (2002): The effect of single strain and traditional mixed strain starter culture on rheological properties of wheat dough and bread quality. Cereal chemistry, 79: 640-647.

Corsetti, A., Gobetti, M., Balestrieri, F., Paoletti, F., Russi, L., Rossi, J. (1998): Sourdough lactic acid bacteria effects on bread firmness and staling. Journal of food science, 63: 347-351.

Crowley, P., Schober, T., Clarke, C., Arendt E. (2002): The effect of storage time on textural and crumb grain characteristics of sourdough wheat bread. European food research and technology, 214: 489-496.

Ganzle, M. G., Vermeulen, N., Vogel, R. F. (2007): Carbohydrate, peptide and lipid metabolism of lactic acid bacteria in sourdough. Food microbiology, 24: 128-138.

Gray, J. A. & Bemiller, J. N. (2003): Bread staling. Molecular basis and control. Comprehensive reviews in food science and food safety, 2: 1-21.

Hammes, W. P. & Gänzle, M. G. (1998): Sourdough breads and related products. In: Microbiology of fermented foods. Vol 1, 2nd Ed, (edited by B.J.B. Wood). Pp. 199-216. London, UK: Blackie Academic & Professional.

Hansen, H. B., Andreasen, M. F., Nielsen, M. M., Larsen, L. M., Bach-Knudsen, K. E., Meyer, A. S., Christensen, L. P., Hansen, A. (2002): Changes in dietary fibre, phenolic acids and activity of endogenous enzymes during rye bread-making. Euro Food Res Technol., 214: 33-42.

Heenan, S. P., Dufour, J. P., Hamid, N., Harvey, W., Delahunty, C. M. (2008): The sensory quality of fresh bread: Descriptive attributes and consumer perceptions. Food Res Int, 41: 989-997.

Kaluđerski, G. & Filipović, N. (1998): Metode ispitivanja kvaliteta žita, brašna i gotovih proizvoda. Tehnološki fakultet, Zavod za tehnologiju žita i brašna (Petrovaradin: Alfa Graf). Novi Sad, Srbija.

Katina K., Heinio, R., Autio, K., Poutanen K. (2006a): Optimization of sourdough process for improved sensory profile and texture of wheat bread. LWT – Food Science and Technology, 39: 1189-1202.

Katina, K., Salmenkallio-Marttila, M., Partanen, R., Forssell, P., Autio, K. (2006b): Effects of sourdough and enzymes on staling of high-fibre wheat bread. LWT - Food Science and Technology, 39: 479-491.

Lambert, J. L., Le-Bail, A., Zuniga, R., Van-Haesendonck, I., Van-Zeveren, E., et al. (2009): The attitudes of European consumers toward innovation in bread;

interest of the consumers toward selected quality attributes. J Sens Stud, 24: 204-219.

Lopez, H. W., Duclos, V., Coudray, C., Krespine, V., Feillet-Coudray, C., Messager, A., Demigné, C., Rémésy, C. (2003). Making bread with sourdough improves mineral bioavailability from reconstituted whole wheat flour in rats. Nutrition, 19: 524-530.

Mrvičić, J., Mikelec, K., Stanzer, D., Križanović, S., Grba, S., Bačun-Družina, V., Stehlik-Tomas, V. (2011): Kiselo tijesto – tradicionalna i prirodna metoda za povećanje kvalitete pekarskih proizvoda. Hrvatski časopis za prehrambenu tehnologiju, biotehnologiju i nutricionizam, 6 (3-4): 89-99.

Oručević Žuljević, S. (2016): Faktori kvaliteta pšeničnog brašna. Poljoprivredno-prehrambeni fakultet Univerziteta u Sarajevu. Sarajevo, B&H.

Plessas, S., Trantallidi, M., Bekatorou, A., Kanellaki, M., Nigam, P., Koutinas, A. A. (2007): Immobilization of kefir and *Lactobacillus casei* on brewery spent grains for use in sourdough wheat bread making. Food Chemistry, 105: 187-194.

Poutanen, K., Flander, L., Katina, K. (2009): Sourdough and cereal fermentation in a nutritional perspective. Food Microbiology, 26: 693-699.

Rehman, S., Nawaz, H., Hussain, S., Ahmad, M. M., Murtaza, M. A., Ahmad, M. S. (2007): Effect of sourdough bacteria on the quality and shelf life of bread. Pakistan journal of nutrition, 6 (6): 562-565.

Symons, L. J. & Brennan, C. S. (2004): The influence of beta-D-glucan rich fractions from barley on the physicochemical properties and in vitro reducing suga release of white breads. J Food Sci., 69: 463-467.

Thiele, C., Gänzle, M. G.,Vogel, R. F. (2002): Contribution of sourdough lactobacilli, yeast and cereal enzymes to the generation amino acids in dough relevant for bread flavour. Cereal Chemistry, 79: 45-51.

SENZORNA SVOJSTVA I BOJA KEKSA U ODNOSU NA INKORPORACIJU JEČMENOG BRAŠNA I TEMPERATURU PEČENJA*

SENSORY AND COLOUR PROPERTIES OF BISCUITS IN RELATION TO BARLEY FLOUR INCORPORATION AND BAKING TEMPERATURE

Amela Džafić[1], Asima Akagić[1], Nermina Spaho[1], Amela Semić[2], Sanja Oručević Žuljević[1]

Original scientific paper

Rezime

Cilj ovog rada je bio ispitati senzorna svojstva i boju keksa proizvedenih sa ječmenim brašnom u odnosu na temperaturu pečenja i udio inkorporiranog brašna ječma.
Pet različitih smjesa integralnog brašna ječma i pšenice (JIB i PIB) su korišteni za proizvodnju keksa: JIB/PIB u kombinacijama: 0/100; 25/75; 50/50; 75/25 i 100/0 prema postupku opisanom u AACC metodi 10-52. Temperature korištene za pečenje su bile 150 i 205 °C tokom 15 i 11 minuta. Proizvedeno je ukupno 10 uzoraka keksa u 2 ponavljanja, dok su sve analize provedene u 3 ponavljanja. Boja je određena kolorimetrom Croma Meter CR 300 (Konica Minolta, Japan). Mjerenja svojstava boje su provedena na površini i dnu keksa. Keksi su ocijenjeni kvantitativnom deskriptivnom analizom (QDA) pomoću ljestvice sa 1-5 bodova na sljedeća svojstva: okus, aroma, topivost i opšta prihvatljivost.
Rezultati su pokazali da je dodatak brašna ječma značajno uticao na promjenu boje i površine i dna keksa, kao i na opštu prihvatljivost, dok na druga senzorna svojstva nije pokazao uticaj. S druge strane temperatura pečenja nije značajno uticala na senzorna svojstva i promjenu boje površine keksa, dok je značajno uticala samo na promjenu boje dna keksa. Senzorna ocjena uzoraka keksa je pokazala da su na ljestvici od 5 bodova svi rezultati bili u rasponu 3.20-4.05, ukazujući da su ovi keksi bili umjereno prihvatljivi. Najbolji senzorni profil je zabilježen kod uzorka sa 75% JIB, pečenog na 150 °C. Isti uzorak je imao najveću promjenu boje površine keksa, dok je najveća promjena boje dna keksa zapažena u uzorku sa 100% JIB, pečenom na 150 °C.

Ključne riječi: *Senzorna svojstva, svojstva boje, ječmeno brašno, temperatura pečenja*

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Faculty of Agriculture and Food Sciences, University of Sarajevo, Bosnia and Herzegovina
[2] Faculty of Health Studies, University "Vitez" Vitez, Bosnia and Herzegovina

Summary

The aim of this study was to examine the sensory and colour properties of biscuits produced with barley flour in relation to baking temperature and share of incorporated barley flour.
Five different mixtures of barley and wheat whole meal flours (BWF and WWF) were used for biscuit production: BWF/WWF in combinations: 0/100; 25/75; 50/50; 75/25 and 100/0 according to procedure described in AACC method 10-52. The temperatures used for baking were 150 and 205°C for 15 and 11 min, respectively. Total of 10 biscuit samples were made in duplicates, while all analysis were performed in triplicates. Colour was determined by the Croma Meter CR 300 (Konica Minolta, Japan). Measurements of colour properties were conducted on upper and lower surface of biscuits. Biscuits were evaluated by Quantitative Descriptive Analysis (QDA) using scale 1-5 scores on 4 properties: taste, aroma, melting and overall acceptability.
Results showed that barley flour incorporation significantly influenced on colour differences of both, upper and lower surface of biscuits and on overall acceptability, while it didn't show influence on other sensory properties. On the other hand, baking temperature didn't significantly influence on sensory properties and on colour differences of upper surface of biscuits, while it significantly influenced only on colour differences of lower surface of biscuits. Sensory evaluation of biscuit samples revealed that in a 5-scores scale, all sensory results were in range of 3.20-4.05 indicating that these biscuits were moderately acceptable. The best sensory profile was observed in sample with 75% BWF, baked on 150°C. The same sample showed the highest colour difference of the upper surface, while the highest colour difference of lower surface was noticed in sample with 100% BWFF, baked on 150°C.

Key words: *Sensory properties, colour properties, barley flour, baking temperature*

INTRODUCTION

By the definition, biscuits and products related to biscuits, are products of certain nutritional and organoleptic properties, obtained from flour as a basic raw material, fats, sugar, starch and other raw materials and additives, by technological procedures of mixing, kneading, whipping, shaping, baking and other procedures (Official Gazette Bosnia and Herzegovina, 2011). Biscuits are popular baked products as food product due to their favorable taste and texture. Their ready to eat nature, availability in a variety of tastes, nutritive value, affordable cost and long shelf life are the reasons of frequent biscuit consumption (Zucco *et al*., 2011; Agama-Acevedo *et al.*, 2012; Hyun-Jung *et al*., 2014; Vitali *et al*., 2009). These products have been suggested as a good way to use composite flours with the aim of improving nutritional value, since they are ready-to-eat, provide a good source of energy, and are consumed widely throughout the world (Arshad *et al.*, 2007).

Barley (*Hordeum sativum* L.) has a long history in food production and in the ancient world was one of the most important food grains. But as alternative food grains, especially wheat, became available, consumption of barley decreased (Newman & Newman, 2006). However, interest in barley as a food grain is currently reviving and there is an increase in new food products with barley, including biscuits, mainly due to the content of health related components (Holtekjølen & Knutsen, 2011; Izydorczyk & Dexter, 2008). Studies have shown that barley grain is an excellent source of soluble and insoluble dietary fibre and other bioactive constituents, such as vitamin E (including tocotrienols), B-complex vitamins, minerals, and phenolic compounds (Madhujith *et al*., 2006; Slavin *et al*., 2000). β-glucans, considered as the major fibre constituents of barley, have been implicated in several health benefits (Behall *et al*., 2006; Cavallero *et al*., 2002; Keenan *et al*., 2007; Li *et al*., 2003). Barley is therefore becoming an important cereal crop from a nutritional and functional point of view.

Currently, barley is increasingly incorporated in already established and new food products either as a whole grain or as a food ingredient (Holtekjølen & Knutsen, 2011). The incorporation of barley in these products is usually aimed at increasing the content of total and soluble fibre in foods, improving their physiological efficacy, and providing health benefits (Brennan & Cleary, 2005). Indeed, significant improvements in the content of dietary fibre can be obtained if barley is incorporated into foods generally made from white wheat flour, which are not considered a good source of fibre. The nutritional value of food with added barley depends on the amount and type of barley added (hull-less or hulled) (Collar & Angioloni, 2014). In addition to providing physiological benefits, the incorporation of different forms of barley changes processing parameters and handling, as well as food texture and some sensory properties, like colour, flavour, and taste. Usually, the barley enriched products have potential to exhibit acceptable sensory properties, especially with incorporation of barley ingredients at low to moderate levels. The incorporation of barley in different share in various wheat-based products including bread, bars, muffins and cookies resulted with sensory scores similar to those for the standard products (Berglund *et al*., 1992). Similar favorable sensory results were reported by Newman *et al*. (1998) for baked products, such as bread, biscuits, sugar cookies and muffins enriched with the barley fibre and by Knuckles *et al*. (1997) for bread with 20% β-glucans barley fractions. Sudha *et al*. (2007) found that biscuits containing 20% barley bran were highly acceptable. Barley flour incorporation improved the colour of the cookies, where the level of 30% was evaluated as the best (Gupta *et al*., 2011). Hassan *et al*. (2012) reported the same level of incorporation as the best in terms of overall acceptability of biscuits enriched with barley meal, and as the barley meal ratio increased, all sensory attributes scores also increased.

Džafić *et al*. (2017) confirmed that barley flour is suitable for the biscuit formula enrichment by improving the nutrition value of the final product due to the increasing β-glucans content and in same time provided acceptable sensory quality.

Baking is one of the most important stages of biscuits production (Siddiqui & Nasreen, 2014). This complex process causes physical, chemical and biochemical changes in the cereal matrix such as crust formation and colour changes (Zanoni *et al.*, 1995; Sablani *et al.*, 1998). Physically, baking involves the simultaneous heat and mass transfer phenomena, and both baking time and temperature are industrially important process considerations affecting the final quality (Fahloul *et al.*, 1994; Sablani *et al.*, 1998). Baking conditions, baking time and temperature highly influence heat transfer and the quality of a final product, due to complex physicochemical interactions that occur between the ingredients, resulting with the difference, primarily in texture, aroma and colour of the products (Shibukawa *et al.*, 1989, Šarić *et al.*, 2014). Colour is one of the most important visual changes occurring during food processing (Surdyk *et al.*, 2004). Among other, the colour is a significant factor that influences purchase decisions of bakery products (Ahmetxhekaj *et al.*, 2016).
Referring to all above mentioned, the aim of this study was to examine the sensory and colour properties of biscuits produced with barley flour in relation to baking temperature and share of incorporated barley flour.

MATERIALS AND METHODS

Whole meal flour samples from organic growing barley provided by Company "Bionatura", Breza and conventionally growing wheat grains obtained from local farmers from growing region Nišići, were used in this study. Blends of wheat and barley whole meal flours (WWF and BWF), obtained by replacing WWF with BWF in different ratios, as shown in Table 1, were used for biscuit production. Biscuits were produced according to the procedure described in AACC (American Association of Cereal Chemists) method 10-52 with modification in sucrose level (reduced from 60% for the standard recipe to 40%) and circular mould used for cutting (5 cm diameter circular mould was used, instead the 6 cm one, required by method). The amount of used flour was calculated based on 14% moisture content. The dough was sheeted to a thickness of 8 mm, cut using a circular mould, placed on a tray and baked at different temperatures (150 and 205 ^{o}C) for different times (15 and 11 min). After baking, biscuits were cooled for 30 min. Total of 10 biscuit samples were prepared in two replicates. Samples were marked on the base of barley flour share (B0, B25, B50, B75 and B100) and used baking temperature (T150 and T205).

Tabela 1. Sadržaj integralnog brašna pšenice i ječma korištenog za pripremu uzoraka keksa na obje temperature pečenja (150 i 205 °C)
Table 1. Content of WWF and BWF used to prepare biscuit samples baked on both temperatures (150 and 205 °C)

Formulations	WWF content %	BWF content %
B0 (control)	100	0
B25	75	25
B50	50	50
B75	25	75
B100	0	100

Colour of fresh cooled biscuits was measured instrumentally using a Minolta Chroma Meter (Konica Minolta, CR 300, Japan). Measurements of colour properties were conducted on upper and lower surface of biscuits. Results were expressed in CIE L*a*b* colour model which is based on the colour perception of 92% of the population that does not have vision deficiencies (Hutchings, 1999; Hutchings *et al.*, 2002). The results were given as the mean values of psychometer light, L*: refers to the lightness of the samples, and ranges from black (L=0) to white (L=100), psychometer tone, a*: participation of red (+) and green (–) colours of components (a negative value of parameter a* indicates green and a positive one indicates red–purple colour) and psychometer chroma, b*: participation of yellow (+) and blue (–) colours of components (positive value of parameter b* indicates yellow while blue indicates negative value) (McGuire, 1992; Popov-Raljić *et al.*, 2013).
Based on the obtained values of L*, a* and b*, additional parameters defining the properties of biscuits colour were calculated. The hue angle, h^o, was calculated by converting the CIEL*a*b* coordinates from rectangular form to polar form and it represents the position of the colour in the spectrum. Chroma, *C**, is the aspect of colour in by which a sample appears to differ from a gray of the same lightness or brightness. The colour saturation, s, is determined by a combination of light intensity and how much it is distributed across the spectrum of different wavelengths. For calculation of mentioned parameters the following equations (Popov-Raljić *et al.*, 2013) were applied:

$$h^o = \tan^{-1}\frac{b*}{a*}$$

$$C* = \sqrt{a^{*2} + b^{*2}}$$

$$s = \frac{C^*}{L^*}$$

The total colour difference (ΔE), in CIE L*a*b* colour model was calculated in relation to colour of control sample with wheat flour, according to the following equation (Purlis *et al.*, 2007):

$$\Delta E = \sqrt{(L^* - L^*_{ref})^2 + (a^* - a^*_{ref})^2 + (b^* - b^*_{ref})^2}$$

Where L^*_{ref}, a^*_{ref}, b^*_{ref} were the reference values taken from control sample with wheat flour.

Sensory evaluation of biscuits by Quantitative Descriptive Analysis (QDA) was conducted to determine the acceptability of the biscuits prepared by BWF incorporation and baked at different temperatures. The organoleptic characteristics of biscuits were determined using a panel of 10 good trained members at Faculty of Agriculture and Food Sciences, University of Sarajevo, B&H.
Selection and training of the panel were done according to recommendations in ISO 8586-1 (1993). The panel members participated in five 1h training and three 30 min evaluation sessions on different days over 4 week. Prior to the assessment, they were trained on various samples of biscuits, made in different combinations of BWF and WWF. The panelists were asked to evaluate the biscuit samples for 4 properties: taste, aroma, melting and overall acceptability using scale 1-5 scores. The obtained sensory data were counted from 10 replicates (panelists were considered as replicate).
All results are expressed as mean ± standard deviation (SD). Two-way analysis of variance with interactions (ANOVA) was used to evaluate whether significant differences existed between the biscuit samples depending on barley flour addition and baking temperature on sensory and color properties. Determined differences were tested by the Tukey test for $p< 0.05$.

RESULTS AND DISCUSSION

The comparison of colour properties among biscuit samples was performed for both, upper and lower surface, and obtained results are presented in Tables 2 and 3.
All colour properties of both, upper and lower surface were significantly influenced by the addition of BWF at all levels. Baking temperature showed significantly influence on all colour properties excepting colour saturation for upper and a* value and hue angle for lower surface.
The lightness of the samples for both, upper (56.56) and lower (50.41) surface, was highest in the case of sample with 25% BWF incorporated, baked at 150 °C. For upper surface this sample was significantly ($P < 0.05$) different from the other samples baked at 150 °C, while for the lower surface it differed only from the samples with 75 and 100% BWF added, baked at 150 °C. No definite trend in increase or decrease in lightness values for both upper and lower surface was observed.
The a* values, with all measurements above zero, for both upper and lower surface, confirm that the red tone is dominating over the green in all biscuit samples. The most expressed red tone of upper and lower surface was observed in the case of addition of 75% BWF, and baking temperature of 150 °C for the upper, and 205 °C for the lower surface. The a* values of both surfaces of biscuits baked at 150 °C significantly ($P < 0.05$) increased with BWF incorporation to 75% level, and then decreased with further increase in the level of BWF.
The b* values, with all measurements high above zero, for both upper and lower surface, confirm that the yellow tone is dominating over the blue in all biscuit

samples, with the yellow tone being more expressed then the red tone. The most expressed yellow tone of upper surface was detected in the sample with 25% BWF added, baked at 205 °C, while the most expressed yellow tone of lower surface had the sample with 25% BWF added, baked at 150 °C. The same samples had the highest value of chroma of upper and lower samples surfaces, respectively.

The lowest value for chroma at the upper surface was detected for the sample containing 50% BWF, baked at 205 °C, while the lowest chroma at the lower surface was detected for the sample with 25% BWF added, baked at 205 °C. Results showed significantly ($P < 0.05$) increase in b* and chroma values of upper surface of biscuits baked at 150 °C with increasing the share of BWF in biscuit formulation.

The hue angle values are in the same area of the hue wheel, the area of light orange colours. Comparing hue values for upper and lower surface, it is obviously that higher hue values were obtained for upper surface, indicating that this surface of samples tends to have more expressed yellow tone. On the other hand, lower hue values for lower surface indicate that this surface of samples tends to have more expressed red tone.

Regarding the colour saturation, the conducted measurements for upper surface confirm that addition of 100% BWF and baking at 205 °C results in the most saturated colour of biscuit samples. While the measurements for the lower surface confirm that addition of 75% BWF and baking at 205 °C results in the same situation.

The total colour difference of biscuit samples for both surfaces was calculated in relation to colour of control samples with wheat flour (Tables 2 and 3). According to the literature (Young & Whittle, 1985; Kim *et al*., 2002) colour differences were categorized as imperceptible differences (0–0.5), slight differences (0.5–1.5), just noticeable differences (1.5–3.0), marked differences (3.0–6.0), extremely marked differences (6.0–12.0) and colours of different shades (above 12.0).

Results showed that BWF incorporation significantly influenced on colour differences of both, upper (Tab. 2) and lower surface (Tab. 3) of biscuits, while baking temperature significantly influenced only on colour differences of lower surface of biscuits.

Marked difference of colour was detected for upper surface of all biscuit samples, excepting sample with 50% BWF incorporated, and baked at 150 °C. The colour difference for upper surface of this sample, together with colour difference for lower surface of samples baked at 205 °C was just noticeable, indicating that these samples had the lowest difference in colour for appropriate surface compared to the control samples with wheat flour. Among the other biscuits, sample with 75% BWF, baked at 150 °C showed the highest colour difference of the upper surface compared to the control sample with wheat flour. In comparison with other biscuits, colour difference for lower surface of sample produced with 100% BWF and baked at 150 °C, was at the level of extremely marked differences. Therefore, this sample had highest difference in colour for lower surface compared to the control sample with wheat flour. The colour difference for lower surface of samples with 25, 50 and 75% BWF added, and baked at 150 °C was at the level of marked difference.

Tabela 2. Svojstva boje površine uzoraka keksa u odnosu na inkorporaciju JIB i temperaturu pečenja

Table 2. Colour properties for upper surface of biscuit samples in relation to BWF incorporation and baking temperature

T	**150 °C**						**205 °C**								
M	**B0**	**B25**	**B50**	**B75**	**B100**	***T***	**B0**	**B25**	**B50**	**B75**	**B100**	***T***	***T***	**M**	***T*xM**
							Upper surface								
L*	54.12 ± 0.79b	56.56 ± 1.63a	53.43 ± 1.46b	49.92 ± 3.04c	50.86 ± 2.13c	x	51.04 ± 2.09ab	51.55 ± 1.63ab	52.13 ± 2.01ab	52.31 ± 2.12a	49.64 ± 2.53b	y	***	***	***
a*	5.64 ± 0.47b	5.75 ± 0.56b	6.65 ± 0.58b	8.14 ± 1.39a	7.70 ± 0.95a	x	5.67 ± 0.76c	6.99 ± 1.09ab	5.18 ± 0.76c	6.04 ± 0.40bc	7.97 ± 0.89a	y	**	***	***
b*	25.87 ± 0.78a	26.04 ± 0.71a	26.14 ± 1.03a	26.76 ± 1.16a	27.17 ± 1.28a	x	24.98 ± 1.37b	27.37 ± 1.13a	24.00 ± 0.94b	24.82 ± 1.08b	27.10 ± 1.28a	y	***	***	***
C*	26.48 ± 0.83b	26.67 ± 0.76b	26.98 ± 1.09ab	27.99 ± 1.50ab	28.24 ± 1.46a	x	25.62 ± 1.49b	28.26 ± 1.33a	24.55 ± 1.05b	25.55 ± 1.13b	28.25 ± 1.38a	y	***	***	***
h°	77.71 ± 0.82a	77.55 ± 1.03a	75.73 ± 0.91b	73.18 ± 2.08c	74.22 ± 1.32bc	x	77.25 ± 1.05ab	75.73 ± 1.67b	77.86 ± 1.36a	76.32 ± 0.41ab	73.63 ± 1.48c	y	*	***	***
s	0.49± 0.02b	0.47 ± 0.02b	0.51 ± 0.02b	0.56 ± 0.06a	0.56 ± 0.04a	x	0.50 ± 0.03b	0.55 ± 0.04a	0.47 ± 0.02b	0.49 ± 0.02b	0.57 ± 0.03a	x	ns	***	***

Different letters in rows from a to b for each parameter indicate significantly different values among mixture at $P < 0.05$.; Different letters in rows from x to y for each parameter indicate significantly different values among temperatures at $P < 0.05$.

Abbreviations: B0, B25, B50, B75, B100 – samples with 0, 25, 50, 75 and 100% BWF added; *T* – temperature; M – mixture; TxM – interaction between temperature and mixture. ns – not significant; * – significant differences at P-value below 0.05; ** – significant differences at P-value below 0.01; *** – significant differences at P-value below 0.001.

Tabela 3. Svojstva boje dna uzoraka keksa u odnosu na inkorporaciju JIB i temperaturu pečenja

Table 3. Colour properties for lower surface of biscuit samples in relation to BWF incorporation and baking temperature

T	**150 °C**						**205 °C**								
M	**B0**	**B25**	**B50**	**B75**	**B100**	***T***	**B0**	**B25**	**B50**	**B75**	**B100**	***T***	***T***	**M**	***T*xM**
							Lower surface								
L*	49.64 ± 2.28a	50.41 ± 2.30a	47.25 ± 2.57ab	45.23 ± 2.50bc	43.37 ± 4.03c	x	49.96 ± 1.57a	50.08 ± 2.01a	40.14 ± 3.72b	38.40 ± 2.25b	49.81 ± 1.95a	y	**	***	***
a*	7.84 ± 1.86b	9.03± 1.50ab	9.61 ± 1.54a	10.32 ± 1.13a	10.08 ± 1.44a	x	6.75 ± 1.24b	6.37 ± 0.30b	12.51 ± 1.07a	13.29 ± 0.60a	7.75 ± 0.48b	x	ns	***	***
b*	28.25 ± 1.57ab	29.69 ± 1.43a	28.66 ± 0.66a	28.60 ± 0.74a	26.91 ± 1.65b	x	28.06 ± 1.52a	27.13 ± 0.60a	28.12 ± 1.94a	27.40 ± 1.38a	28.31 ± 0.80a	y	**	*	***
C*	29.35 ± 1.97bc	31.06 ± 0.79a	30.26 ± 0.93ab	30.43 ± 0.66ab	28.79 ± 1.19c	x	28.87 ± 1.76b	27.87 ± 0.60b	30.80 ± 1.89a	30.47 ± 1.10a	29.35 ± 0.77ab	y	*	***	***
h°	74.64 ± 2.79a	73.13 ± 2.58ab	71.51 ± 2.62bc	70.15 ± 2.22c	69.36 ± 3.68c	x	76.56 ± 1.65a	76.78 ± 0.62a	65.98 ± 2.13b	64.07 ± 1.96b	74.67 ± 1.05a	x	ns	***	***
s	0.59 ± 0.06c	0.62 ± 0.04bc	0.64 ± 0.05ab	0.67 ± 0.04a	0.67 ± 0.05ab	x	0.58 ± 0.04a	0.56 ± 0.02a	0.77± 0.04a	0.79 ± 0.03a	0.59 ± 0.02a	y	*	***	***

Different letters in rows from a to b for each parameter indicate significantly different values among mixture at $P < 0.05$.; Different letters in rows from x to y for each parameter indicate significantly different values among temperatures at $P < 0.05$.

Abbreviations: B0, B25, B50, B75, B100 – samples with 0, 25, 50, 75 and 100% BWF added; *T* – temperature; M – mixture; TxM – interaction between temperature and mixture. ns – not significant; * – significant differences at P-value below 0.05; ** – significant differences at P-value below 0.01; *** – significant differences at P-value below 0.001.

Tabela 4. Promjena boje površine i dna uzoraka keksa u odnosu na inkorporaciju JIB i temperaturu pečenja

Table 4. Colour differences for upper and lower surface in relation to BWF incorporation and baking temperature

T	**150 ºC**					**205 ºC**							
M	**B25**	**B50**	**B75**	**B100**	***T***	**B25**	**B50**	**B75**	**B100**	***T***	***T***	**M**	***T*xM**
Upper surface	3.00 ± 1.32ab	2.16 ± 0.80b	5.18 ± 3.64a	4.33 ± 2.09ab	x	4.66 ± 1.01a	3.34 ± 2.20a	3.30 ± 1.46a	4.75 ± 1.79a	x	ns	*	*
Lower surface	5.64 ± 1.51 ab	3.46 ± 1.81b	5.57 ± 1.54ab	7.59 ± 5.68a	x	2.35 ± 0.33a	1.79 ± 0.53a	2.34 ± 0.34a	2.53 ± 1.13a	y	***	**	ns

Different letters in rows from a to b for each parameter indicate significantly different values among mixture at $P < 0.05$.; Different letters in rows from x to y for each parameter indicate significantly different values among temperatures at $P < 0.05$.

Abbreviations: B25, B50, B75, B100 – samples with 25, 50, 75 and 100% BWF added; *T* – temperature; M – mixture; TxM – interaction between temperature and mixture. ns – not significant; * – significant differences at P-value below 0.05; ** – significant differences at P-value below 0.01; *** – significant differences at P-value below 0.001.

Tabela 5. Senzorna ocjena uzoraka keksa u odnosu na inkorporaciju JIB i temperaturu pečenja

Table 5. Sensory evaluation of biscuit samples in relation to BF incorporation and baking temperature

T	**150 ºC**						**205 ºC**								
M	**B0**	**B25**	**B50**	**B75**	**B100**	***T***	**B0**	**B25**	**B50**	**B75**	**B100**	***T***	***T***	**M**	***T*xM**
Taste	3.70 ± 0.95 a	4.00 ± 0.53 a	3.95 ± 0.50 a	3.95 ± 0.69 a	3.60 ± 0.94 a	x	3.45 ± 0.69 a	3.65 ± 0.67 a	3.80 ± 0.59 a	3.80 ± 1.03 a	3.50 ± 0.97 a	x	ns	ns	ns
Aroma	3.60 ± 0.84 a	3.85 ± 0.58 a	3.75 ± 0.42 a	4.05 ± 0.76 a	3.30 ± 0.95 a	x	3.55 ± 0.69 a	3.60 ± 0.52 a	3.60 ± 0.52 a	3.60 ± 0.97 a	3.25 ± 0.86 a	x	ns	ns	ns
Melting	3.70 ± 1.06 a	3.95 ± 0.69 a	3.70 ± 0.48 a	4.05 ± 0.76 a	3.65 ± 0.94 a	x	3.10 ± 0.88 a	3.60 ± 0.70 a	3.50 ± 0.71 a	3.90 ± 0.88 a	4.00 ± 0.82 a	x	ns	ns	ns
General acceptability	3.70 ± 0.67 a	3.85 ± 0.47 a	4.00 ± 0.47 a	4.05 ± 0.76 a	3.20 ± 0.92 a	x	3.50 ± 0.71 a	3.20 ± 0.63 a	3.75 ± 0.63 a	3.85 ± 0.94 a	3.20 ± 0.92 a	x	ns	*	ns

Different letters in rows from a to b for each parameter indicate significantly different values among mixture at $P < 0.05$.; Different letters in rows from x to y for each parameter indicate significantly different values among temperatures at $P < 0.05$.

Abbreviations: B0, B25, B50, B75, B100 – samples with 0, 25, 50, 75 and 100% barley flour added; *T* – temperature; M – mixture; TxM – interaction between temperature and mixture. ns – not significant; * – significant differences at P-value below 0.05; ** – significant differences at P-value below 0.01; *** – significant differences at P-value below 0.001.

The results of the conducted evaluation of sensory properties of biscuits point out the differences between samples with different share of BWF incorporated, and samples baked at different temperatures.

According to the two-way ANOVA baking temperature showed no significance for total sensory quality, while BF incorporation showed significance only for general acceptability (Tab 5). There was no interaction between these factors for sensory quality.

In general the best sensory profile was observed in the case of sample with 75% BWF incorporated, baked at 150 °C. It had a more intense aroma, as well as melting, and showed the highest score for general acceptability. On the other hand, the samples containing only WWF and BWF, baked at 205°C, had the weakest sensory properties.

Sensory evaluation (taste, aroma, melting, and overall acceptability) of biscuit samples revealed that in a 5-scores scale, all samples were evaluated in average from 3.10 to 4.05 scores, indicating that these biscuits were moderately acceptable.

CONCLUSIONS

The here presented results showed that BWF incorporation significantly influenced on all colour properties and on colour differences of both, upper and lower surface of biscuits. In addition, barley provides acceptable sensory quality. The best quality according sensory evaluation showed sample produced from 75% BWF, baked at 150°C.

ACKNOWLEDGEMENTS

This study was part of the Programme in Higher Education, Research and Development in the Western Balkans 2012-2014 the Agriculture Sector (HERD/Agriculture) “Antioxidant activity and stability of bioactive components during processing of certain raw materials of plant origin Bosnia and Herzegovina”.

REFERENCES

AACC, Approved Methods of Analysis, 10th Ed. Method 10-52, Baking Quality of Cookie Flour—Micro Method, Approved October 15, 1997, American Association of Cereal Chemists, St. Paul, MN, 1997.

Agama-Acevedo, E., Islas-Hernández, J. J., Pacheco-Vargas, G., Osorio-Díaz, P., Bello-Pérez, L. A. (2012): Starch digestibility and glycemic index of cookies partially substituted with unripe banana flour. LWT-Food Sci. Technol. 46 (1), 177-182.

Ahmetxhekaj, S., Koceva Komlenić, D., Lukinac Čačić, J., Jukić, M., Hrg, D., Budžaki, S. (2016): The temperature profile during cookie baking as a function of sugar granulation. Technologica acta, 9(1), 65–76.

Arshad, M. U., Anjum, F. M., Zahoor, T. (2007): Nutritional assessment of cookies supplemented with defatted wheat germ. Food Chem. 102, 123–128.

Behall, K. M., Scholfield, D. J., Hallfrisch, J. G. (2006): Barley β-glucan reduces plasma glucose and insulin responses compared with resistant starch in men. Nutr. Res. 26, 644–650.

Berglund, P. T., Fastnaught, C. E., Holm, E. T. (1992): Food uses of waxy hull-less barley. Cereal Foods World, 37(7), 707–714.

Brennan, C. S., Cleary, L. J. (2005): The potential use of cereal (1→3,1→4)-β-D-glucans as functional food ingredients. J. Cereal Sci. 42, 1–13.

Cavallero, A., Empilli, S., Brighenti, F., Stanca, A. M. (2002): High (1→3,1→4)-β-Glucan Barley Fractions in Bread Making and their Effects on Human Glycemic Response. J. Cereal Sci. 36, 59–66.

Collar, C., Angioloni, A. (2014): Nutritional and functional performance of high β-glucan barley flours in breadmaking: Mixed breads versus wheat breads. Eur. Food Res. Technol. 238, 459–469.

Džafić, A., Oručević Žuljević, S., Spaho, N., Akagić, A. (2017): Effects of barley flour addition and baking temperature on β-glucans content and biscuit properties. Technologica Acta. 10 (1), 1-56.

Fahloul, D., Trystram, G., Duquenoy, A., Barbotteau, I. (1994): Modeling heat and mass transfer in band oven biscuit baking. Lebensmittel Wissenchauft und Technologie, 27, 119–124.

Gupta, M., Bawa, A. S., Abu-Ghannam, N. (2011): Effect of barley flour and freeze-thaw cycles on textural nutritional and functional properties of cookies. Food Bioprod. Process. 89, 520–527.

Hassan, A. A., Rasmy, N. M., Foda, M. I., Bahgaat, W. K. (2012); Production of Functional Biscuits for Lowering Blood Lipids. World Journal of Dairy & Food Sciences 7, 1–20.

Holtekjølen, A. K., Knutsen, S. H. (2011): Antioxidant activity and phenolics in breads with added barley flour. In: Preedy, V.R., Watson, R.R., Patel, V.B. (Eds), Flour and breads and their fortification in health and disease prevention. Academic Press, Elsevier. London, Burlington, San Diego, 355-363.

Hutchings, J. (1999): Food color and appearance, 2nd ed. Aspen Publishers, Inc. Gaithersburg, MD

Hutchings, J., Luo, R., Ji, W. (2002): Calibrated color imaging analysis of food, in: Color in Food, B.D. MacDougall. Woodhead Publishing in Food Science and Technology. Cambridge, England, 352–366.

Hyun-Jung, C., Cho, A., Seung-Taik, L. (2014): Utilization of germinated and heat-moisture treated brown rices in sugar-snap cookies. LWT-Food Sci. Technol. 57 (1), 260-266.

ISO 8586:2014 (2014): Sensory analysis, General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors

Izydorczyk, M. S., Dexter, J. E. (2008): Barley β-glucans and arabinoxylans: Molecular structure, physicochemical properties, and uses in food products-a Review. Food Res. Int. 41, 850–868.

Keenan, J. M., Goulson, M., Shamliyan, T., Knutson, N., Kolberg, L., Curry, L. (2007): The effects of concentrated barley beta-glucan on blood lipids in a population of hypercholesterolaemic men and women. Br. J. Nutr. 97, 1162–8.

Kim, S., Park, J., Hwang, I. K. (2002): Quality attributes of various varieties of Korean red pepper powders (*Capsicum annuum* L.) and color stability during sunlight exposure. J.Food Sci. 67, 2957–2961.

Knuckles, B. E., Hudson, C. A., Chiu, M. M., Sayre, R. N. (1997): Effect of β-glucan barley fractions in high-fiber bread and pasta. Cereal Foods World 42, 94–99.

Li, J., Kaneko, T., Qin, L.-Q., Wang, J., Wang, Y. (2003): Effects of barley intake on glucose tolerance, lipid metabolism, and bowel function in women. Nutrition 19, 926–929.

Madhujith, T., Izydorczyk, M., Shahidi, F. (2006): Antioxidant properties of pearled barley fractions. J. Agric. Food Chem. 54, 3283–3289.

McGuire, R. G. (1992): Reporting of Objective Color Measurements. Hortscience, 27, 1254-1255.

Newman, C. W., Newman, R. K. (2006): A brief history of barley foods. Cereal Foods World. doi:10.1094/CFW-51-0004

Newman, R. K., Ore, K. C., Abbott, J., Newman, C. W. (1998): Fiber enrichment of baked products with a barley milling fraction. Cereal Foods World 43, 23–25.

Official gazette Bosnia and Herzegovina (2011): Regulations of biscuits and products related to biscuits. NN 51/11.

Popov-Raljić, J. V., Mastilović, J. S., Laličić-Petronijević, J. G., Kevrešan, Ž. S., Demin, M. A. (2013): Sensory and color properties of dietary cookies with different fiber sources during 180 days of storage. Hem. Ind. 67 (1) 123–134.

Purlis, E., Salvadori, V. O. (2007): Bread browning kinetics during baking. J. Food Eng. 80 (4), 1107-1115.

Sablani, S. S., Marcotte, M., Baik, O. D., Castaigne, F. (1998): Modeling of simultaneous heat and water transport in the baking process. Lebensmittel Wissenchauft und Technologie 31, 201–209.

Šarić, B., Nedeljković, N., Šimurina, O., Pestorić, M., Kos, J., Mandić, A., Sakač, M., Šarić, Lj., Psodorov, Đ., Mišan, A. (2014): The influence of baking time and temperature on characteristics of gluten free cookies enriched with blueberry pomace. Food and Feed Research 41 (1), 39-46.

Shibukawa, S., Sugiyama, K., Yano, T. (1989): Effects of heat-transfer by radiation and convection on browning of cookies at baking. Journal of Food Science 54 (3), 621-624.

Slavin, J., Marquart, L., Jacobs, D. (2000): Consumption of whole-grain foods and decreased risk of cancer: proposed mechanisms. Cereal Foods World 45(1), 54–58.

Sudha, M. L., Vetrimani, R., Leelavathi, K. (2007): Influence of fibre from different cereals on the rheological characteristics of wheat flour dough and on biscuit quality. Food Chem. 100, 1365–1370.

Surdyk, N., Rosén, J., Andersson, R., Åman, P. (2004): Effects of asparagine,

fructose, and baking conditions on acrylamide content in yeast-leavened wheat bread. J. Agr. Food Chem. 52 (7), 2047-2051.
Vitali, D., Dragojević, I. V., Šebečić, B. (2009): Effects of incorporation of integral raw materials and dietary fibre on the selected nutritional and functional properties of biscuits. Food Chem. 114, 1462–1469.
Young, K. W., Whittle, K. J. (1985): Color measurement of fish minces using Hunter *L, a, b* values. J. Sci. Food Agr. 36, 383–392.
Zanoni, B., Peri, C., Bruno, D. (1995): Modelling of browning kinetics of bread cut during baking. Lebensm.-Wiss. Technol. 28, 604-609.
Zucco, F., Borsuk, Y., Arntfield, S. D. (2011): Physical and nutritional evaluation of wheat cookies supplemented with pulse flours of different particle sizes. LWT-Food Sci. Technol. 44 (10), 2070-2076.

IMPACT OF THE TEMPERATURE ON THE MILK ABSORPTION OF DIFFERENT KINDS OF BREAKFAST CEREALS*

UTICAJ TEMPERATURE NA APSORPCIJU MLIJEKA RAZLIČITIH VRSTA ŽITNOG DORUČKA

Munevera Begić[1], Jasmina Tahmaz[1], Amila Mulagić[1], Amela Semić[2], Sanja Oručević Žuljević[1]

Original scientific paper

Rezime

Cilj rada bio je ispitati uticaj temperature na apsorpciju mlijeka različitih vrsta žitnog doručka. Za izvođenje ogleda korišteno je šest uzorka žitnog doručka (kukuruzne pahuljice CS1, pšenične pahuljice CS2, ječmene pahuljice CS3, čokoladni musli CS4, *musli 5 vrsta voća* CS5, musli sa dodatkom voća CS6). Na ispitivanim uzorcima određen je sadržaj vlage, sadržaj pepela, udio dodataka (CS4, CS5, CS6), apsorpcija mlijeka na temperaturi od 20 °C i 40 °C, i senzorna ocjena. Najmanji sadržaj vlage je imao ekspandirani uzorak žitnog doručka dok su uzorci sa pahuljicama i uzorci sa različitim dodacima imali veći sadržaj vlage. Najveći sadržaj pepela je imao ekspandirani uzorak žitnog doručka (CS1). Apsorpcija mlijeka je bila veća sa povećanjem vremena potapanja i bila je veća sa mlijekom na 40 °C osim kod uzorka *musli 5 vrsta voća* kod kojeg je apsorpcija bila veća sa mlijekom na 20 °C. Ekspandirani uzorak je imao najveću ukupnu senzornu ocjenu i najveću hrskavost. Utvrđena je visoka korelacija između svih ispitivanih uzoraka u pogledu apsorpcije na 20 °C i 40 °C u vremenskom intervalu.

Ključne riječi: *žitni doručak, temperatura, mlijeko, apsorpcija.*

Summary

The purpose of this research was to examine the effect of the temperature on the absorption of milk of different kinds of cereal breakfast. Six samples of cereal breakfast were used for this experiment (corn flakes CS1, wheat cereals CS2, barley cereals CS3, chocolate muesli CS4, muesli *5 kinds of fruits CS5* and tropic muesli CS6). The following analyses were determined: moisture, content of ash, proportion of

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina

[1]Faculty of Agriculture and Food Sciences, University of Sarajevo, Zmaja od Bosne 8, 71 000 Sarajevo, Bosnia and Herzegovina
[2]Faculty of Health Studies, University "Vitez" Vitez, Bosnia and Herzegovina
Correspondence: Munevera Begić, Faculty of Agriculture and Food Sciences, University of Sarajevo, Bosnia and Herzegovina, e-mail: m.begic@ppf.unsa.ba

fruit and chocolate (CS4, CS5, CS6), absorption of milk at the temperature of 20 °C and 40°C and sensory evaluation. The expanded sample of cereal breakfast had the lowest concentration of moisture content while the samples with rolled cereal and with fruit and/or chocolate added had higher moisture. The corn flakes sample (CS1) had the highest concentration of ash. The absorption of milk increased during the immersion and was higher at 40 °C except for the muesli *5 kinds of fruits* where the absorption was higher at 20 °C. Corn flakes sample of cereal breakfast had the highest sensory total score and the highest crispness. A high degree of correlation was determined among all analyzed samples related to milk absorption of 20 °C and 40 °C in time interval.

Key words: *cereal breakfast, temperature, milk, absorption.*

INTRODUCTION

Cereal based food is the main in diet for the majority of the population around the world. The extrusion and a hydrothermal processing of the cereals are thermal methods of processing that are applied in order to improve nutritive, hygienic and physical-chemical characteristics of the raw material (Filipović *et al.,* 2003). Ready-to-eat (RTE) breakfast cereals are processed grain formulations suitable for human consumption without further cooking in the home. They are relatively shelf-stable, lightweight, and convenient to ship and store. They are made primarily of corn, wheat, oats, or rice, in about that order of the quantities produced, usually with added flavor and fortifying ingredients (Fast, 2000). What they have in common is that contain large amounts of starch, that significantly change the properties during the extrusion, and in that way they influence the quality and texture of a made product. The type and composition of raw ingredients play an import role in extrusion cooking by affecting the frictional forces, and therefore the resulting polymer structures (Faller *et al.,* 2000). Other variables in the extruder also depend on the characteristics of the raw materials such as: the pressure, the temperature, and the engine load (Guy, 2001). The kind of food we eat for breakfast is usually healthier and more nutritive, unlike the other meals we eat during the day (Ruxton, 2016). Ready-to-eat breakfast cereals are favored by consumers of all ages because of their convenience, variety, and high nutritional value (Hegenbart, 1995).

Most ready-to-eat (RTE) breakfast cereals may be grouped into 12 general categories for discussion of their manufacturing processes: 1) flaked cereals (corn flakes, wheat flakes, and rice flakes), including extruded flakes, 2) gun-puffed whole grains, 3) extruded gun-puffed cereals, 4) shredded whole grains, 5) extruded and other shredded cereals, 6) oven-puffed cereals, 7) granola cereals, 8) extruded expanded cereals, 9) baked cereals, 10) compressed flake biscuits, 11) muesli-type products, and 12) filled bite-size shredded wheat (Fast, 2000). The cereals are crispy when consumed, they are used as a supplement in chocolate, some candy products, for making the mixtures with an extruded – expanded cereals (Bešlagić, 2005). Extrusion-cooked direct expanded breakfast cereals have cellular-like structures mainly formed by air pockets

surrounded by walls of gelatinized starch that contribute to texture and hydration capacity (Solomon, 2014). Breakfast cereals are commonly consumed by soaking in milk (Sacchetti *et al.*, 2003) where the product readily takes up moisture resulting in undesirable changes, especially in the most desirable and primary quality attributes like crispiness, brittleness, and crunchiness due to the plasticization effect of water (Machado *et al.*, 1999; Sacchetti *et al.*, 2003; Gondek and Lewicki, 2006). The loss of crispiness in extruded cereals prepared with milk can be described as a rapid, unbalanced two-way mass transfer, where one part of the ingredients from the surface of the product dissolves in milk, and one part of water from milk penetrates inside the product (Jukić *et al.*, 2010). Breakfast cereal consumption is associated with diets high in vitamins and minerals and low in fat (grade B) but is not associated with increased intakes of total energy or sodium (grade C) or risk of dental caries (grade B). Oat- or barley-based cereals can help to decrease cholesterol concentrations (grade A), and high-fiber, wheat-based cereals can improve bowel function (grade A). Regular breakfast cereal consumption is associated with a lower body mass index and less risk of being overweight or obese (grade B). Presweetened breakfast cereals do not increase the risk of overweight and obesity in children (grade C). Whole-grain or high-fiber breakfast cereals are associated with a lower risk of diabetes (grade B) and cardiovascular disease (grade C). There is emerging evidence of associations with feelings of greater well-being and a lower risk of hypertension (grade D), but more research is required (Williams, 2014).
The main goal of this paper was to examine influence of the milk temperature on absorption and sensory quality of different kinds of cereal breakfast.

MATERIAL AND METHODS

Six different cereal breakfast samples purchased from local market were used (Tab. 1) for this study.

Table 1. An overview of the analyzed samples

Ord. No.	Sample	Description from the declaration	Producer	Mark
1.	Corn flakes	Corn flakes	Lubella, Poland	CS1
2.	Wheat cereals	Rolled wheat grain	Biona & Beyon, Serbia	CS2
3.	Barley cereals	Rolled barley grain	Schapfen Muhle, Germany	CS3
4.	Chocolate muesli	Oat, corn and wheat cereals, chocolate chips	Mestemacher, Germany	CS4
5.	Muesli 5 kinds of fruits	Oat, corn, wheat, barley and rye cereals, dry fruit (hazelnut, raisins, banana, apple, apricot) and sunflower grains	Mestemacher, Germany	CS5
6.	Muesli tropic	Wheat, barley, oat, rye and soy cereals, dry fruit (banana, pineapple, raisins, mango, papaya)	Štark, Serbia	CS6

Moisture (drying at 105 °C until constant mass), and ash content (burning at 600 °C until constant mass) were determined (Kaluđerski and Filipović, 1998). Non-cereal parts in samples CS4, CS5 and CS6 were separated and weighed. Proportions of added components (fruit and chocolate) in samples were expressed in %. Three measurements per each sample were made and mean values with standard deviations (S. D.) reported.

Milk absorption examination

Examination of the absorption of milk was done according to the method of Jukić *et al.*, (2010). Knowing the mass of the sample before and after the absorption and the content of a dry matter enabled the counting of absorption intensity. The first step was weighing 4 g of the sample in 10 laboratory glasses of 100 ml.

After that 40 ml of milk at 20 °C were added to each glass. The same procedure was done with milk at 40 °C. The first five probations were filtered out through the strainer after each minute from 1-5 minutes. The remaining 5 probations were filtered after 10, 15, 20, 25 and 30 minutes. The filtered sample was weighed after that. The milk absorption of the cereal breakfast was calculated according to the following formula:

$$\mathbf{A = (m_{\text{filtered sample}} - m_{\text{dry matter of the sample}})/m_{\text{dry matter of the sample}}\ [g/g\ d.m.]}$$

Where:

A = the absorption of milk (g/g d.m.)

m = the mass (g)

Sensory evaluation of the samples

Sensory evaluation of the samples was determined using a panel of 7 well trained members and included the following characteristics: appearance, the texture and crispness, smell, taste, general acceptability and harmoniousness. For sensory evaluation, the hedonic scale was used. The scale was consisted of 9 gradations, where the beginning value (1) means the maximum unpleasant impression, while the highest value means the maximum pleasant impression (Radovanović and Popov–Raljić, 2001). The obtained sensory data were counted from 7 replicates (panelists were considered as replicate).

For the presentation of the results a descriptive statistics was used and the correlation. The interpretation of the correlation was done according to Grujić and Spaho (2010), where r from ± 0.00 do ± 0.20 means no or insignificant correlation, r from ± 0.20 to ± 0.40 weak correlation, r from ± 0.40 to ± 0.70 significant correlation and r from ± 0.70 to ± 1.00 high or very high correlation.

RESULTS AND DISCUSSION

The results of the moisture, ash and content of the supplements (CS4, CS5 and CS6) are shown in Table 2.

Table 2. Moisture, ash and non-cereal supplements in samples

	CS1	CS2	CS3	CS4	CS5	CS6
Moisture (%)	4.42 ± 0.38	10.44 ± 0.31	9.46 ± 0.03	8.12 ± 0.35	8.49 ± 0.59	6.63 ± 0.08
Ash (%)	1,71 ± 0.06	1.23 ± 0.93	1.40 ± 0.71	1.22 ± 0.93	1.55 ± 0.21	1.68 ± 2.77
Supplements (%)	-	-	-	15.51 ± 0.07	15.20 ± 0.85	22.65 ± 2.33

The content of moisture in examined samples ranged from 4.42% (CS1) to 10.44% (CS2). CS1 had the lowest content of moisture (4.42%), while the samples with different supplements had higher content of moisture (CS4, CS5, CS6) compared to the other samples. The content of ash in tested samples ranged from 1.22% (CS4) to 1.71% (CS1). In terms of the ash content CS1 had the highest value (1.71%), while the samples with the supplements added CS5 and CS6 showed closely values (1.55 and 1.68%, respectively). The sample CS6 with tropical fruit had the highest portion of the supplements (22.65%), while the chocolate muesli and *muesli 5 kinds of fruits* had lower portion of the supplements 15.51% and 15.20%, respectively.

Table 3. Milk absorption during 30 min (g/g d.m.)

	1 min	2 min	3 min	4 min	5 min	10 min	15 min	20 min	25 min	30 min
CS1 a	1.26± 0.13	1.72± 0.03	1.90± 0.07	1.98± 0.03	2.03± 0.07	2.41± 0.04	2.78± 0.01	3.09± 0.26	3.07± 0.06	3.79± 0.31
CS1 b	1.46± 0.10	1.78± 0.01	1.97± 0.00	2.02± 0.07	2.09± 0.07	2.62± 0.14	2.91± 0.22	3.24± 0.19	3.12± 0.05	3.39± 0.20
CS2 a	0.89± 0.07	0.97± 0.05	0.98± 0.04	1.00± 0.09	1.10± 0.04	1.25± 0.08	1.31± 0.10	1.34± 0.07	1.58± 0.15	1.57± 0.14
CS2 b	0.80± 0.01	0.83± 0.00	0.84± 0.15	1.10± 0.09	1.00± 0.05	1.12± 0.07	1.48± 0.20	1.39± 0.03	1.79± 0.32	1.58± 0.12
CS3 a	0.82± 0.13	0.86± 0.19	0.97± 0.21	0.86± 0.12	0.88± 0.08	0.97± 0.17	0.99± 0.08	1.30± 0.05	1.30± 0.22	1.28± 0.14
CS3 b	0.75± 0.16	0.76± 0.00	0.82± 0.01	0.85± 0.05	0.82± 0.02	0.95± 0.01	1.09± 0.03	1.05± 0.03	1.29± 0.09	1.25± 0.01
CS4 a	0.73± 0.08	0.78± 0.07	0.95± 0.04	0.82± 0.06	0.91± 0.04	1.10± 0.07	1.33± 0.05	1.37± 0.02	1.63± 0.16	1.72± 0.15
CS4 b	0.76± 0.18	0.86± 0.11	0.89± 0.19	0.91± 0.02	1.04± 0.02	1.08± 0.04	1.17± 0.08	1.30± 0.07	1.24± 0.14	1.44± 0.27
CS5 a	0.69± 0.01	1.09± 0.05	0.88± 0.17	1.07± 0.10	1.04± 0.13	1.10± 0.00	1.31± 0.06	1.35± 0.14	1.26± 0.14	1.21± 0.11
CS5 b	0.66± 0.11	0.83± 0.01	0.76± 0.08	0.93± 0.08	0.94± 0.10	1.07± 0.12	1.09± 0.02	1.29± 0.18	1.07± 0.00	1.22± 0.06
CS6 a	0.88± 0.11	0.81± 0.18	0.99± 0.10	0.74± 0.01	0.94± 0.15	1.09± 0.18	1.04± 0.35	1.20± 0.06	1.43± 0.09	1.39± 0.42
CS6 b	0.78± 0.16	0.92± 0.03	1.00± 0.18	0.90± 0.34	1.03± 0.20	1.32± 0.00	1.47± 0.22	1.12± 0.15	1.24± 0.02	1.66± 0.12

*a - milk absorption at 20°C, b - milk absorption at 40°C

Absorption values in all samples were increasing during immersion (Tab. 3) of immersion in milk (Tab. 3). Variations in the absorption can be seen during immersion in samples CS2 and CS3. Moreover, the values of the absorption of those samples are smaller when compared to the other samples probably because of their hardness and inability to absorb milk. The highest values at both temperatures of the absorption were found in the expanded sample CS1 probably because of its crispness and the porous structure that allowed fast absorption of milk. Milk absorption was higher at 40 °C in all samples (Tab. 3). The exception was the sample CS5 that had higher values of the milk absorption at 20 °C.
However, milk absorption of sample with chocolate chips (CS4) were higher at 40 °C in the first 10 minutes of testing and after this time the values of the absorption were higher at 20 °C. According to obtained results temperature and immersion duration are affected differently on examined samples depending of the kind of samples and added supplements. For the CS1 absorption at 40 °C is recommended but with less time immersion. Samples of rolled cereal (CS2, CS3) behaved similarly during immersion at both temperatures. In addition, absorption of samples with fruit and chocolate chips (CS4, CS5, and CS6) varied in great extent depending on the kind of supplements added.

Table 4. The results of the samples sensory evaluation

	CS1	CS2	CS3	CS4	CS5	CS6
Appearance	7.14 ± 0.00	6.57 ± 0.40	6.14 ± 0.20	5.86 ± 0.00	6.21 ± 0.30	5.86 ± 0.20
Texture and crispness	7.71 ± 0.40	5.71 ± 0.20	6.07 ± 0.30	6.21 ± 0.10	5.71 ± 0.40	5.93 ± 0.30
Smell	7.00 ± 0.20	6.43 ± 0.40	5.79 ± 0.10	6.64 ± 0.10	6.07 ± 0.10	6.00 ± 0.40
Taste	7.57 ± 0.20	6.07 ± 0.10	6.00 ± 0.40	6.89 ± 0.20	6.21 ± 0.70	6.64 ± 0.10
General acceptability	7.50 ± 0.10	6.43 ± 0.40	5.93 ± 0.30	6.64 ± 0.30	6.21 ± 0.30	6.29 ± 0.20
Harmoniousness	6.50 ± 0.10	6.71 ± 0.20	6.50 ± 0.10	6.36 ± 0.10	6.14 ± 0.20	6.21 ± 0.10

Sample CS1 had the highest score for appearance (7.14) while the lowest score had samples CS4 and CS6 (5.86). The expanded sample CS1 was the best evaluated in terms of the texture and crispness (7.71), and the lowest evaluated were the samples CS2 and CS5 (5.71). For the sensory property of the smell the best evaluated was the sample CS1 (7.00), while the lowest evaluated was CS3 (5.79). The taste of the tested samples of breakfast cereals was the best evaluated in the sample CS1 (7.57), while the lowest evaluated was the sample CS3 (6.00). In terms of general acceptability the best evaluated sample was CS1 (7.50) and the lowest evaluated was the sample CS3 (5.93). The harmoniousness was the best evaluated in the sample CS2 (6.71) while the lowest evaluated was the sample with 5 kinds of fruits CS5 (6.14). The sample with rolled cereal CS2 was the best evaluated and had not contained any supplements, while the sample CS5 had many different supplements which probably resulted with a low score in term of harmoniousness. The best evaluated sample was the expanded sample CS1 with a total average grade of 7.24 with no any supplements added. The lowest evaluated was the sample with rolled cereal CS3 with a total average grade of 6.07 probably because of its hardness that was prominent in this sample. In the same time the crispness of this sample was reduced which was resulting in low score.

Table 5. The correlation of the milk absorption (g/g d.m.) and immersion time

	1 min	*2 min*	*3 min*	*4 min*	*5 min*	*10 min*	*15 min*	*20 min*	*25 min*	*30 min*
1 min	1									
2 min	0.92	1								
3 min	0.97	0.96	1							
4 min	0.91	0.98	0.94	1						
5 min	0.94	0.98	0.97	0.98	1					
10 min	0.95	0.97	0.98	0.97	0.99	1				
15 min	0.92	0.96	0.96	0.98	0.98	0.98	1			
20 min	0.94	0.97	0.97	0.98	0.98	0.97	0.97	1		
25 min	0.95	0.91	0.95	0.95	0.95	0.95	0.96	0.97	1	
30 min	0.92	0.93	0.97	0.94	0.97	0.97	0.97	0.96	0.97	1

In terms of the correlation between milk absorption and the time of immersion all samples showed a very high correlation (r=0.91 to 1.00) on both evaluated temperature (Tab. 5).

CONCLUSIONS

The study has shown that the value of milk absorption was in high correlation with the temperature of milk and the time interval of the immersion. It also depended on the kind of breakfast cereals (corn flakes or rolled cereal) and different non-cereal supplements added.

REFERENCES

Bešlagić, S. (2005): „Technology of confectionery products“. Arka PRESS, Sarajevo, Bosnia and Herzegovina.

Faller, J. F., Faller, J. Y., Klein, B. P. (2000): „Physical and sensory characteristic of extruded corn/soy breakfast cereals“. Journal of Food Quality 23, pp: 87-102.

Fast, B. R. (2000): „Breakfast Cereals and How They Are Made Second Edition“. American Association of Cereal Chemists, pp: 14-57.

Filipović, S., Sakač, M. B., Ristić, M. D., Kormanjoš, Š. M., Galić, S., Ivanišević, S. (2003): „Thermic methods of cereals production". Magazine for process techniques and energetics in agriculture PTEP, 7(1-2), pp: 3-7.

Gondek, E., Lewicki P. P. (2006): „Antiplasticization of cereal-based products by water". Part II. Breakfast cereals. Journal of Food Engineering 77:644–652.

Grujić, S., Spaho N. (2010): „Needs of the consumers and the quality of food products". Faculty of Agriculture and Food Sciences" University of Sarajevo. Bosnia and Herzegovina.

Guy, R. (2001): „Extrusion cooking: Technologies and applications". Woodhead Publishing in Food Science and Technology. England.

Hegenbart, S. H. (1995): „Mastering the morning: Creating breakfast cereals". Food Product Design 5(4), pp: 27-51.

Jukić, M., Ugarčić- Hardi Ž., Koceva Komlenić D., Gorički Z., Kuleš A. (2010): „Textural characteristics of ready-to-eat breakfast cereals produced from different types of cereal and with varying water addition during extrusion process". Croatian Journal of Food Science Technology, pp: 1-10.

Kaluđerski, G., N. Filipović (1998): „Methods of testing grain quality, flour and ready products". Faculty of Technology, The institute for technology of grain and flour quality, Novi Sad, Serbia.

Machado, M. F., Oliveira, F. A. R., Cunha L. M. (1999): „Effect of milk fat and total solid concentration on the kinetics of moisture uptake by ready-to-eat breakfast cereal". Int. J. Food Sci. Technol. 34:47–57.

Radovanović, R., Popov-Raljić J. (2001): "Sensory analysis of food products". Faculty of Technology, Novi Sad. Serbia.

Ruxton, C. (2016): „The benefits of breakfast and breakfast cereals". European Breakfast Cereal Association, pp: 1-16.

Sacchetti, G., Pittia, P., Biserni, M., Pinnavaia, G. G., Rosa M. D. (2003): „Kinetic modeling of textural changes in ready-to-eat breakfast cereals during soaking in semi-skimmed milk". Int. J. Food Sci. Technol. 38:135–143.

Solomon, W. K. (2014): „Hydration Kinetics of Direct Expanded *tef* Flour Breakfast Cereals in Water and Milk". Food Science & Nutrition 2.1 (2014): pp: 39–45.

Williams, P. G. (2014): „The Benefits of Breakfast Cereal Consumption: A Systematic Review of the Evidence Base". American Society for Nutrition. Advances in Nutrition 5: 636S–673S.

INFLUENCE OF DRYING AND SOAKING IN NaCl, CITRIC ACID AND OREGANO SOLUTIONS ON POTATO CHIPS QUALITY*

UTICAJ SUŠENJA I URANJANJA U RASTVORE NaCl-a, LIMUNSKE KISELINE I ORIGANA NA KVALITET ČIPSA OD KROMPIRA

Jasmina Tahmaz[1], Aida Mostić[1], Amina Šenderović[1], Munevera Begić[1], Nermina Đulančić[1], Sabina Operta[1], Amila Semić[1]

Original scientific paper

Summary

The aim of this study was to investigate application of different pretreatments, including soaking in different solutions with and without additional drying before frying. After peeling and slicing, potato slices were soaked in following solutions: (1) NaCl, (2) NaCl+citric acid and (3) NaCl+citric acid+oregano (*Origanum vulgare)* at 60°C during 60 minutes. After soaking samples were divided into two parts – one part was fried immediately at 170°C, another part was dried at 50°C for 30 minutes, and fried after drying. It is believed that soaking before frying could influence many quality properties like taste, color, crispness and fat uptake.
Samples pretreated in salt solution had the lightest color, highest level of NaCl and best taste. Drying pretreatment caused decreasing of moisture, higher NaCl content and generally improved all sensorial properties (especially crispness, color and taste). The highest level of moisture content, darkest color and worst sensorial properties was noticed in samples soaked in oregano solution without additional drying.

Key words: *potato chips, soaking, drying, NaCl, crispness, sensorial properties*

Rezime

Cilj rada je ispitati primjenu različitih predtretmana uključujući uranjanje u različite rastvore sa i bez dodatnog sušenja prije prženja. Nakon ljuštenja i rezanja, listovi krompira su se uranjali u rastvore 1) NaCl-a, 2) NaCl-a i limunske kiseline, i 3) NaCl-a, limunske kiseline i sušenog začinskog origana (*Origanum vulgare)* na 60°C u trajanju od 60 minuta. Nakon uranjanja uzorci su podijeljeni na dva dijela: jedan dio se odmah pržio na 170°C, a drugi dio je prije prženja sušen 30 minuta na 50°C. Smatra se da uranjanje prije prženja može uticati na mnoge osobine čipsa (okus, boja, hrskavost i sadržaj masti).

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Faculty of Agriculture and Food Sciences, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

Uzorci predtretirani u rastvoru soli imali su najsvjetliju boju, najveći sadržaj NaCl-a i najbolji okus. Predtretman sušenjem je doveo do dodatnog sniženja sadržaja vlage, povećanja sadržaja NaCl-a i generalno bolje senzorne osobine (psebno hrskavost i okus). Najveći sadržaj vlage, najtamniju boju i najgore ocijenjene senzorne osobine su imali uzorci koji su se uranjali u rastvor origana.

Ključne riječi: *čips od krompira, uranjanje, sušenje, NaCl, limunska kiselina.*

INTRODUCTION

Fried foods are among the favorites for people around the world. Some fried products could contain high amount of fat due to indivertible fat absorption during deep-fat frying. Nowadays, consumers are more interested in health related and functional food products. Optimal frying conditions are very important to produce quality potato chips with low fat content. During deep-fat frying, water evaporates and moves out of the food. The migration of water occurs during deep fat frying by following order – at first water evaporates from crust and after from core to surface/crust of product, because of difference in water concentration between core and surface. As water vapor leaves from surface of product, it voids for the fat to enter later (Pedreschi *et al*., 2007).
There are several studies related to application of different pretreatment techniques with purpose to improve quality of potato chips. The several pre-frying procedures have been proposed for many reasons of quality improvement e.g. to reduce the oil absorption, amount of acrylamide, and also to get better texture, color, taste, crispness and overall acceptability (Pedreschi *et al*., 2007). Studies related to soaking pretreatment mostly includes solutions of NaCl, citric, ascorbic, acetic acid, tomato juice, different plant extracts or their combination. Abou - Zaid (2015) managed to improve sensorial quality by soaking of chips in tomato juice with added NaCl and citric acid. El – Shafaw *et al*., (2014) applied soaking in cloves and rosemary extracts to significantly decrease acrylamide in chips. Citric acid is usually used in potato technology as antioxidant for inhibition of browning of potato slices (Calder *et al*., 2011; Tsouvaltzis and Brecht, 2016).
Many authors managed to decrease acrylamide in chips using pretreatments by soaking in solutions of NaCl, citric acid (Basuny and Arafat, 2013, Abou - Zaid, 2015; Bunger *et al*., 2003), $CaCl_2$ (Khalaf *et al*., 2015) and tomato juice (Abou – Zaid, 2015) or their combinations. According to Gaikwad and Athmaselvi (2016) potato chips pretreated in indifferent concentrations of NaCl and citric acid solutions had better sensorial properties. Soaking before frying caused considerably better color in French fries (Burch *et al*., 2008). The similar results were reported by Ismial *et al*. (2013) meaning that soaking before frying resulted in better and lighter color in French fries.
Besides soaking pretreatments, many studies showed positive effects of drying pretreatments on acrylamide reduction (Pedreschi *et al*., 2007; Kampuse *et al*., 2014) and fat reduction in potato chips or French fries., but sensorial properties like

color and texture were also improved. Abdulla *et al*. (2013) reported that drying method in combination with soaking in citric acid solution improved sensorial properties of sweet potato chips. Drying of raw potato slices leads to better moisture evaporation and can reduce frying time.

The aim of this study was to investigate the effects of different pretreatment methods by soaking in different solutions (NaCl, citric acid and oregano) and additional pretreatments on quality of fried potato chips. Due to former literature reports, soaking and drying pretreatments were performed with the purpose to improve the chemical and sensorial properties of fried potato chips. According to adjusted goals, authors put the hypothesis that different pretreatments would influence the potato chips quality parameters (chemical and sensorial).

MATERIALS AND METHODS

Potatoes (*Solanum tuberosum*), frying oil (Zvijezda, Croatia), soaking agents: table salt (Solana, Tuzla), dried powdered oregano (*Origanum vulgare)* were obtained from the local market in Sarajevo, Bosnia and Herzegovina.

Potatoes were washed in tap water and peeled. The slicing was performed using kitchen peeling knife to the thickness of about 1.5- 1.8 mm. Slices were rinsed in distilled water to eliminate starch from surface after slicing.

Then, the slices were divided into three batches of about 160g of potato slices for each. Then each batch was additionally divided into two parts (80g). Each part was soaked for 60 min in 200 mL of the different solutions at temperature of 60°C. After soaking a part of each pretreated samples was drained to eliminate water from surface and fried immediately at 170°C for 5 minutes. Another part was dried at 50°C for 30 minutes in laboratory oven and fried at 170°C for 3 minutes. Frying was performed in kitchen fryer (N DF 10, Neo). The following samples were obtained:

1- S: Soaked in 3% NaCl solution and fried at 170°C for 5 minutes
2- S”: Soaked in 3% NaCl solution, dried at 50°C for 30 minutes and fried at 170°C for 3 minutes
3- S+CA: Soaked in 1 % NaCl + 0.1% citric acid solution and fried at 170°C for 5 minutes
4- S+CA”: Soaked in 1 % NaCl + 0.1% citric acid solution, dried at 50°C for 30 minutes and fried at 170°C for 3 minutes
5- S+CA+O: Soaked in 1 % NaCl + 0.1% citric acid + 1% powdered oregano solution and fried at 170°C for 5 minutes
6- S+CA+O”: Soaked in 1 % NaCl + 0.1% citric acid + 1% powdered oregano solution, dried at 50°C, 30 minutes and fried at 170°C for 3 minutes.

Fat content was determined by Soxhlet extraction method with diethyl ether p.a. for 6 hours. The moisture was determined gravimetrically by drying of about 3 g of each representative samples in laboratory oven at 105°C for 2.5 h (AOAC, 2000). Determination of NaCl was performed by Mohr titration using 0.1M $AgNO_3$ and 5% potassium chromate as indicator (E.K. 8045, JUS., 1993).

Sensorial evaluation was done using 5-point hedonic scale (5- excellent/extremely acceptable, 4-good/acceptable, 3- neither like nor dislike, 2–dislike slightly but satisfy, 1-dislike extremely/extremely unacceptable). Semi trained panelists (n=12) were selected for sensory analysis. The samples were evaluated for color, texture/crispness, odor, taste and overall acceptability. The scores received by each evaluated sample were averaged and compared. The panelists were asked to put comments for each evaluated sample.

All determinations were carried out in triplicate and data were reported as mean ± standard deviation. Influence of soaking agents was tested using one-way ANOVA, and significant differences ($p<0.05$) were calculated using Tukey test. Significant influence of drying pretreatment was evaluated using t-test ($p<0.05$).

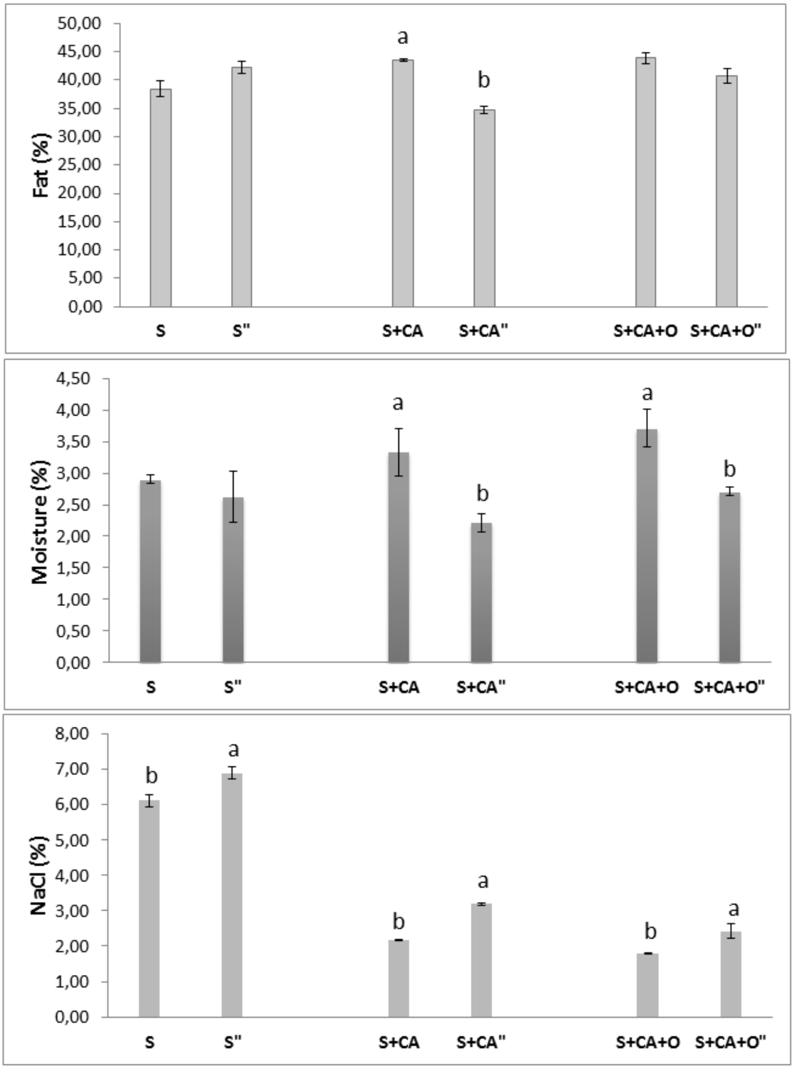
RESULTS AND DISCUSSION

Comparison between dried and non-dried soaked samples

Results of chemical analysis are given in Fig. 1. The highest level of moisture content was noticed in chips soaked in oregano solution without additional drying (3.71%), and the lowest in chips soaked in citric acid solution with drying pretreatment (2.21%). Fat content in all samples was pretty high (34.72—43.93%). The lowest fat content was noticed in sample pretreated in NaCl + citric acid solution and dried before frying. Drying pretreatment decreased fat content significantly in samples with citric acid for about 8% (S+CA vs. S+CA”). The highest fat content was noticed in sample with oregano without drying (S+CA+O). Samples soaked in NaCl had slightly higher fat content after drying pretreatment. In S+CA+O” sample fat was slightly decreased in comparison to S+CA+O.

All samples pretreated by drying had lower moisture content. Because of that, those samples had better crispness. Drying pretreatments decreased moisture content in all soaked samples, but the differences were significant only for S+CA and S+CA+O samples. The decreasing of moisture after drying was the smallest in the samples soaked in 3% salt solution. But, it is interesting that this sample (S) had lower moisture content comparing to other non-dried samples (Fig.1), and consequently better crispness. Drying pretreatment caused additional moisture evaporation prior frying, which resulted to lower final moisture content, better crispness and better the other sensorial properties. High moisture content is undesirable in potato chips because the moisture decreases crispness.

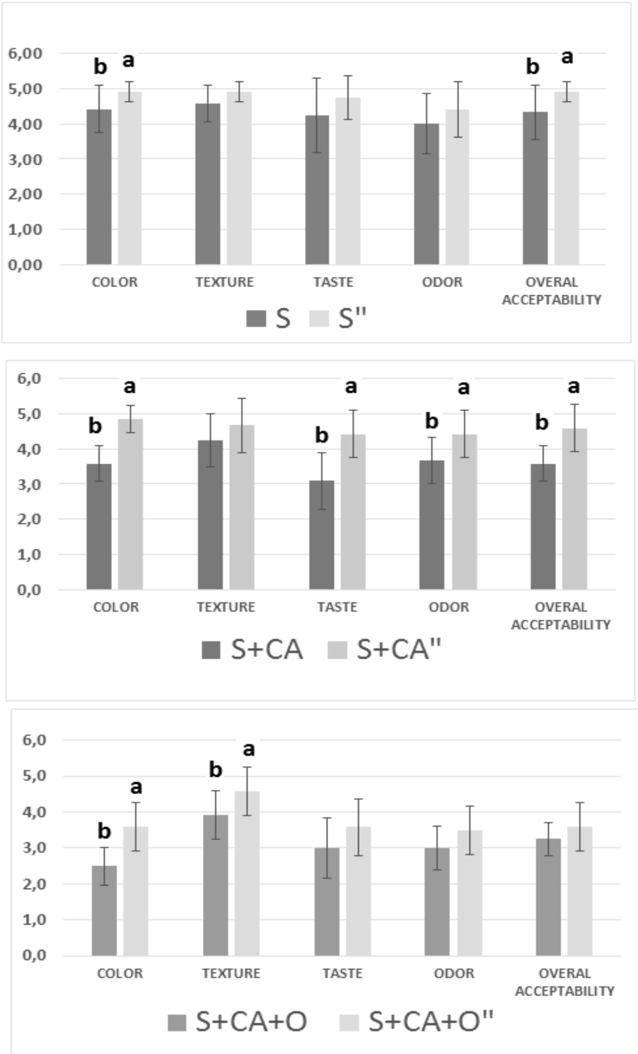
These results are in agreement with data reported by other authors for similar samples (Khalaf *et al.*, 2015; Tahmaz and Smajić, 2006; Tahmaz, 2005; Souci *et al.*, 1990). Kampuse *et al.* (2014) studied influence of different drying pretreatments on moisture, fat and texture of French fries. It was found that drying pretreatments had tendency to decrease moisture content after frying (but not significantly), and also to decrease fat content significantly. In comparison with control the lowering of fat content in dried samples amounted about 2-6% (Kampuse *et al.*, 2014). The highest decreasing in fat content was notices in samples soaked in in citric acid solution (8.84%) and oregano solution (3.18%).



Different small letters represent significant differences between samples with and without additional drying (p≤0.05)

Figure 1. Chemical analysis of chips samples pretreated by soaking in solutions of: S - 3% NaCl; S"- 3% NaCl and dried before frying; S+CA -1% NaCl + 0.1% citric acid; S+CA"- 1 % NaCl+0.1% citric acid and dried before frying; S+CA+O - 1% NaCl+0.1% citric acid+1% oregano; S+CA+O"-1 % NaCl + 0,1% citric acid+1% oregano and dried before frying

Slika 1. Hemijske analize uzoraka čipsa (predtretirani uranajnjem u rastvore: S- 3% NaCl-a ; S"- 3% NaCl-a i sušeni prije prženja; S+CA - 1%NaCl-a+0,1% limunske kiseline; S+CA"- 1%NaCl-a+0,1% limunske kiseline i sušeni prije prženja; S+CA+O -1% NaCl-a +0,1%+limunske kiseline+1% origana; S+CA+O": 1% NaCl-a+0,1%+limunske kiseline+1% origana i sušeni prije prženja



Different small letters represent significant differences between samples with and without additional drying (p≤0.05)

Figure 2. Sensorial evaluation of potato chips pretreated by soaking in solutions of: S - 3% NaCl; S"- 3% NaCl and dried before frying; S+CA -1% NaCl + 0.1% citric acid; S+CA"- 1 % NaCl+0.1% citric acid and dried before frying; S+CA+O - 1% NaCl+0.1% citric acid+1% oregano; S+CA+O"-1 % NaCl + 0,1% citric acid+1% oregano and dried before frying

Slika 2. Senzorna ocjena uzoraka čipsa predtretiranih uranjanjem u rastvore: S- 3% NaCl-a ; S"- 3% NaCl-a i sušeni prije prženja; S+CA - 1%NaCl-a+0,1% limunske kiseline; S+CA"- 1%NaCl-a+0,1% limunske kiseline i sušeni prije prženja; S+CA+O -1% NaCl-a+0,1%+limunske kiseline+1% origana; S+CA+O": 1% NaCl-a +0,1%+limunske kiseline+1% origana i sušeni prije prženja

Results of sensorial evaluation are given in Fig. 2. As it was expected, the highest level of NaCl was in samples soaked in 3% NaCl solution. However, all samples pretreated by drying had significantly higher NaCl content. Drying pretreatment caused significantly increased salt content, which was expected. Due to better moisture evaporation, the better concentration of total dry mater had occurred and therefore the salt concentration increased. The normal content of salt in industrial chips is in the range 0.57 – 2.40 % (Soucci *et al*., 1999; Tahmaz and Smajić, 2006). Although literature data showed lower NaCl content, samples with high salt content (S -6.12% and S" – 6.89%) were evaluated as preferable (Fig. 2, Tab. 1) for taste (4.25 – good and 4.83 - excellent) and overall acceptability (S – good (4.33) and S" – excellent (4.92)). This phenomenon could be explained by consumer preferences or habits to consume salty chips. The lowest salt content was noticed in sample soaked in oregano solution without drying (1.80%). This sample also had the lowest scores for taste and overall acceptability.

According to sensory evaluation all dried samples had better evaluated crispness (not significantly), which could be explained by lower moisture content. Generally, all sensorial properties were better evaluated in dried samples. In try to explain this, it could be considered that higher moisture loss probably caused better acceptability of all sensorial attributes. The aroma and taste compounds were better concentrated in samples with lower moisture content (better evaluated taste and odor). Also, as it was noticed before, the crispness is in negative correlation with moisture, and crispness has very huge importance among other sensorial properties in snacks.

Comparison between different soaking pretreatments

ANOVA showed significant influence of soaking agent for fat and NaCl content in both types of samples (dried and non-dried before frying).

In all cases the highest moisture content was in samples soaked in oregano solution. The differences in moisture content between soaking pretreatments were significant only in samples without additional drying. Chips soaked in 3% NaCl solution had significantly lower fat content in comparison to other samples (pretreatments without drying), but significantly higher comparing to citric acid soaked samples (pretreatment with additional drying). The samples soaked in 3% NaCl solution had significantly higher level of NaCl. The lowest NaCl content was noticed in chips soaked in oregano solution (significantly in both cases – dried and non-dried).Obtained results were expected, due to higher concentration of NaCl in soaking solution for S and S" samples in comparison to samples soaked in other solutions.

The best evaluated were samples soaked in NaCl for all sensorial properties (Fig. 2, Tab. 1). Samples soaked in oregano solution were evaluated with the lowest scores for all sensorial properties. Markedly bad sensorial properties were noticed in sample soaked in citric acid solution without drying. In pretreatments without additional drying, chips samples soaked in citric acid and oregano solutions had significantly lower scores for taste and overall acceptability than salt soaked sample. Samples soaked in oregano (with and without drying) and in citric acid without additional drying had darker color. Because of that the color of these samples were evaluated with lower scores. The darkest color and lowest scores were noticed in oregano soaked sample without additional drying. Samples soaked in NaCl solution had the lightest color, and its color was evaluated from acceptable (without drying) to excellent (with drying).

Table 1. The most common comments and scores for overall acceptability given by panelists

Tabela 1. Najčešći komentari i ocjene za ukupnu prihvatljivost dodijeljeni tokom senzorne ocjene

Sample / *uzorak*	Comments / *komentari*	Overall acceptability scores / *broj bodova za ukupnu prihvatljivost*	Overall acceptability – description / *opisna ocjena ukupne prihvatljivosti*
S	Very salty, very light color, oily taste and oily appearance, excellent	4.33±0,78	Acceptable - good
S+CA	Very sour taste	3.58±0,45	Acceptable - good
S+CA+O	Smells like fish, not salty enough	3.25±0,45	Neither like nor dislike
S“	Excellent	4.92±0,29	Excellent
S+CA“	Sour taste	4.25±0,87	Acceptable - good
S+CA+O“	Not salty enough, dark non-attractive color	3.58±0,67	Acceptable - good

S – soaked in 3% NaCl solution and fried, S+CA – soaked in 1% NaCl+0,1% citric acid solution and fried, S+CA+O – soaked in 1%NaCl+0,1% citric acid+1% oregano solution and fried, S” – soaked in 3% NaCl solution, dried at 50°C and fried, S+CA” – soaked in 1% NaCl + 0,1% citric acid solution, dried at 50°C and fried, S+CA+O” – soaked in 1% NaCl+ 0,1% citric acid+1% oregano solution, dried at 50°C and fried

S – predtretiran uranjanjem u 3%-tni rastvor NaCl-a i pržen; S+CA - predtretiran uranjanjem u rastvor NaCl-a (1%) i limunske kiseline (0,1%) i pržen S+CA+O - predtretiran uranjanjem u rastvor NaCl-a (1%), limunske kiseline (0,1%) i origana (1%) i pržen; S”- predtretiran uranjanjem u 3%-tni rastvor NaCl-a i sušen prije prženja;; S+CA”- predtretiran uranjanjem u rastvor NaCl-a (1%) i limunske kiseline (0,1%) i sušen na 50°C prije prženja;; S+CA+O”- predtretiran uranjanjem u rastvor NaCl-a (1%), limunske kiseline (0,1%) i origana (1%) i sušen prije prženja

Soaking pretreatment did not significantly influence evaluation of texture. The scores for texture were similar for all samples, but slightly higher in dried samples because of better moisture loss. In the term of the different soaking agents, it can be seen that soaking in 3% NaCl in all samples gave better texture. The worst texture/crispness was in samples soaked in oregano, which could be explained with the highest moisture level in all oregano samples.

Chemical properties (low moisture and high salt level) and sensorial properties (good color, taste, texture) of chips soaked in high concentrated NaCl solution could be

explained by osmosis process. Due to osmosis, NaCl molecules attend to migrate from solution to potato slices and amount of water also migrate from potato slices to soaking solution. Because of that after soaking moisture decrease and NaCl increase
According to overall acceptability it can be seen that samples were mostly evaluated as acceptable/good. It is also very important to say that neither sample was evaluated as unacceptable/not satisfy (Tab. 1). Also, the panelists were asked to put the comment for each sample and the common comments are given in Tab. 3. It can be seen that NaCl soaked chips were marked as very salty (or even over salty). But nevertheless, these very salty chips samples were evaluated better in comparison with other soaked samples and recognized as excellent. Pretty low scores for citric acid soaked samples could be explained by sour taste. Considering the given comments low scores for oregano soaked samples were not unexpected because of nonspecific and unpleasant fish like odor/aroma, poorly salty taste and dark non-attractive color) (Tab. 1). It is strongly expected that potato chips poses following characteristics: to be crispy, salty and oily. The fat in snacks (together with low moisture and good crispness) contributes to better texture and mouth feel. High fat content in chips commonly can cause better solvation in mouth. Crispness and solvation could be marked as advanced textural properties of potato chips. The high salt content additionally can contribute to this mouth feeling (solvation).
Samples soaked in NaCl solution were more acceptable than blanched samples (Gaikwad and Athmaselvi, 2016). Bunger *et al.* (2003) reported that French fries pretreated by soaking in 2% NaCl solution had better texture, color and overall acceptability than control. According to Ismial *et al.* (2013) soaking in salt and citric acid solutions improved color and taste of potato chips.

CONCLUSION

- Drying after soaking leaded to high moisture loss, better crispness, taste and overall acceptability in all samples.
- Drying pretreatment could be suggested to industrial application because of higher moisture loss, improved crispness, color and overall acceptability.
- The best sensorial acceptability was noticed in samples pretreated in 3% NaCl solution with additional drying, which could be explained by consumer preferences to salty chips.
- Generally the worst sensorial properties were noticed in samples soaked in oregano solution. Because of that application of oregano as soaking agent could not be suggested for future practical application.
- Soaking in different concentration of NaCl solution could be suggested for future research and possible industrial application.
- Different pretreatments had influences on sensorial and chemical properties of potato chips samples. Statistical analysis (t test, $p \leq 0.05$) proved that dying before frying leaded to significant:

  - decreasing of moisture in citric acid (S+CA vs. S+CA”) and oregano (S+CA+O vs. S+CA+O”) soaked samples
  - decreasing of fat in citric acid soaked samples (S+CA and S+CA”)
  - increasing of NaCl content in all kind of soaked samples
  - improvement of color in all soaked samples
  - improvement of overall acceptability in citric acid (S+CA vs. S+CA”) and oregano (S+CA+O vs. S+CA+O”) soaked samples
  - improvement of taste and odor in citric acid soaked samples and also improvement of texture in oregano soaked samples
- ANOVA (t test, $p \leq 0.05$) proved that soaking in NACl (S and S”) had improved had improved color, taste and overall acceptability in samples pretreated without additional drying. Also ANOVA showed significantly ($p \leq 0.01$) better color, odor, taste and overall acceptability in NaCl and oregano soaked samples (S” and S+CA”) in comparison to oregano soaked chips (S+CA+O”).

REFERENCES

Abdulla, G., El-Shourbagi, A. G., Sitohi, M. Z. (2014): Effect of Pre-drying, Blanching and Citric Acid Treatments on the Quality of Fried Sweet Potato Chips, *American Journal of Food Technology*, 9, (1): 39-48.

Abou-Zaid, F. O. F. (2015): The effect of using some treatments on reduction of acrylamide formation in processed potatoes, Agri-Industrialization Unit, Plant Production Department, Desert Reasearch Center, Cairo, Egypt

A.O.A.C. (2000): Association of Official Method of Analysis. Official Analytical Chemists 17^{th} Ed., Gaithersburg, MD: AOAC International.

Basuny, A. M. M., Arafat, S. M. (2013): Reduction of acrylamide formation, polymer compounds and oil uptake in potato slices during frying. *Current Research in Microbiology and Biotechnology*, Vol. 1, No. 3 (2013): 111-118

Bunger, A., Moyano, P., Rioseco, V. (2003): NaCl soaking treatment for improving the quality of French-fried potatoes. *Food Res Int* 36:161–166

Burch, R. S., Trzesicka, A., Clarke, M., Elmore, J. S., Briddon, A., Matthews, W., Webber, N. (2008): The effects of low-temperature potato storage and washing and soaking pre-treatments on the acrylamide content of French fries. *J. Sci. Food Agric.*, 88: 989–995.

Calder, B. L., Kash, E. A., Davis-Dentici, K. B. (2011): Comparison of sodium acid sulfate to citric acid to inhibit browning of fresh-cut potatoes. *J Food Sci.76(3).*

E. K. 8045, JUS, (1993), Određivanje sadržaja natrijum-hlorida, Službeni list SRJ 27/93

El-Shawaf, M. G, El-Zamzamy, F. M., Mekky, T. M. (2014): Reduction of Acrylamide Formation in Potato Chips Using Natural Antioxidants Sources from Plant Extracts, *Middle East Journal of Agriculture Research*, 3(1): 89-99.

Gaikwad, K. Y., Athmaselvi, K. A. (2016): Effect of soaking pre-tretments on different characteristics of fried potato chips, Department of Food Process Engineering, School of Bioengineering, Food Safety and Quality Management, SRM University, Chennai, India

Ismial, S. A.-M. A., Ali, R. F. M., Samy, W. M. (2013): Impact of pre-treatments on the acrylamide formation and organoleptic evolution of fried potato chips, *American Journal of Biochemistry and Biotechnology*, 9 (2): 90-101.

Kampuse, S., Jefimovs, A., Rakcejeva, T. (2014): The influence of pre-treatment method on the fat content decrease in French fries, Department of Food Technology, Faculty of Food Technology, Latvia University of Agriculture, Jelgava, Latvia

Khalaf, H. H. A., Sharoba, A. M., El-Desouky, A. I., El-Bassiony, K. R. A., Afifi, S. A. M. (2015): Effect of some pre-treatments on acrylamide concentration in potato chips, *Annals of Agric. Sci*.Vol. 53(2), ISSN 1110-0419). 211–220.

Mestdagh, F., De Wilde, T., Fraselle, S., Govaert, Y., Ooghe, W. Degroodt, J. M., Verhe R., Van Peteghem, C. and De Meulenear, B. (2008): Optimization of the blanching process to reduce acrylamide potatoes. *LWT-Food Science and Technology, 41(9)*

Pedreschi, F., Leo´n, J., Mery, D., Moyano, P., Pedreschi, R., Kaack, K., Granby, K. (2007): Color development and acrylamide content of pre-dried potato chips, *Journal of Food Engineering 79* (2007) 786–793.

Souci, S. W., Fachmann, W., Kraut, H. (1990): Food composition and nutrition tables, Wissenschaftliche verlagsgesellschaft mbH, Sttutgart.

Tahmaz, J. (2005): Proizvodnja i kvalitet čipsa i srodnih proizvoda, Magistarski rad, Poljoprivredni fakultet Univerziteta u Sarajevu, Sarajevo.

Tahmaz, J**.**, Smajić, A. (2006): Extruding process characteristics and differences between extruded and non-extruded potato snacks, *Works of Faculty of Agriculture-University of Sarajevo*, No.58/2.

Tsouvaltzis, P., Brecht, JK. (2016): Inhibition of Enzymatic Browning of Fresh-Cut Potato by Immersion in Citric Acid is Not Solely Due to pH Reduction of the Solution, *Journal of food processing and preservation* 9 (2): 90-101.

POTATO CHIPS QUALITY PRETREATED BY SOAKING IN GINGER, PAPRIKA AND CURRY SOLUTIONS*

KVALITET ČIPSA OD KROMPIRA PREDTRETIRANOG URANJANJEM U RASTVORE ĐUMBIRA, PAPRIKE I KARIJA

Jasmina Tahmaz[1], Amina Šenderović[1], Aida Mostić[1], Munevera Begić[1], Nermina Đulančić[1], Sabina Operta[1], Mersiha Alkić-Subašić[1]

Original scientific paper

Summary

The aim of this study was to investigate the different soaking pretreatments on quality of potato chips. Experiment was performed on chips samples prepared by soaking of potato slices in different salty (1% NaCl) solutions with addition of powdered paprika, ginger and curry spice mixture before frying. The potato slices were soaked at 60°C for 60 minutes, and then fried at 170°C. A part of each sample was additionally pretreated by drying at 50°C for 30 minutes and then fried. One sample was blanched without soaking and fried.

The best sensorial acceptability was noticed in samples pretreated in curry and ginger solutions. The darkest color and worst sensorial properties were noticed in blanched sample. Ginger soaked sample had the lightest and most acceptable color. But, neither sample was evaluated as unacceptable.

Soaked samples had significantly ($p \leq 0.05$) lower moisture content, higher NaCl content, mostly better color, better crispness and overall acceptability. Unlike moisture, the fat content did not decrease in soaked samples. Drying after soaking caused better moisture loss, better crispness and overall acceptability in all soaked samples.

Key words: *potato chips, soaking, curry, paprika, ginger*

Rezime

Cilj ovog rada je ispitati različite predtretmane i njihov uticaj na kvalitet čipsa. Istraživanja su rađena na uzorcima čipsa koji su se prije prženja uranjali u 1%-tne rastvore soli (NaCl-a) sa dodatkom mljevene paprike, đumbira i mješavine začina za kari. Uranjanje je trajalo 60 minuta na 60°C, nakon čega je slijedilo prženje za dio od svakog uzorka. Drugi dio je nakon uranjanja podvrgnut sušenju na 50°C u trajanju od

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina

[1] Faculty of Agriculture and Food Sciences, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

30 minuta, a nakon toga su prženi. Jedan uzorak je prije prženja bio blanširan bez predtretmana uranjanjem.
Najbolje senzorne osobine su imali uzorci tretirani u rastvorima karija i đumbira. Najtamniju boju i najslabije ocijenjene senzorne osobine je imao blanširani uzorak. Uzorak predtretiran u rastvoru đumbira je imao najsvjetliju i najbolje ocijenjenu boju. Nijedan uzorak nije ocijenjen kao neprihvatljiv.
Uzorci predtretirani uranjanjem su imali značajno ($p \leq 0.05$) niži sadržaj vlage, viši sadržaj soli, bolju hrskavost i opštu prihvatljivost u odnosu na blanširani. Za razliku od vlage, sadržaj masti se nije uspio sniziti kod predtretiranih uzoraka. Predtretman sušenjem nakon uranjanja i prije prženja je doveo do snižavanja sadržaja vlage, poboljšanja hrskavosti i drugih senzornih osobina.

Ključne riječi: *čips od krompira, uranjanje, kari, so, paprika, đumbir*

INTRODUCTION

Deep fat frying process is nowadays widely used in food preparation. This kind of preparation means that food is completely immersed in hot frying oil and all food surface area is surrounded with hot boiling oil. Many factors (e.g. frying temperature, kind of oil, duration of frying process, moisture in food) influence the quality of fried food and fat uptake. Boiling temperature of oil is higher in comparison with boiling point of water. Because of that, when oil boils water also boils and evaporate. Frying can be described as process in which water evaporates from food and oil is absorbed in food. As water vapor leaves from surface of product, it voids for the fat to enter later. One of the most popular industrial produced food products is potato chips. Optimal frying conditions have great importance in production of quality potato chips and French fries with low fat content (Fellows, 2000; Mehta and Swinburn, 2001; Moreira, 2007).
Application of different pretreatment techniques before frying can be suggested for purpose of potato chips quality improvement. Pretreatment methods usually include drying, baking and soaking processes before frying. According to Ismial *et al.* (2013), Abou - Zaid (2015), Gaikwad and Athmaselvi (2016) and Bunger *et al.* (2003) managed to improve quality of potato chips by soaking in different concentrations of NaCl solution. Abou - Zaid (2015) improved sensorial quality by soaking of chips in tomato juice with added NaCl and citric acid. Many studies showed that content of acrylamide in chips could be decreased using pretreatments by soaking in solutions of NaCl, citric acid (Basuny and Arafat, 2013, Abou - Zaid, 2015; Bunger *et al.*, 2003; Calder *et al.*, 2011; TsouvaltzIs and Brecht, 2016), $CaCl_2$ (Khalaf *et al.*, 2015), L-asparaginase (Onishi *et al.*, 2015), tomato juice (Abou – Zaid, 2015) or their combinations. El – Shafaw *et al.* (2014) applied soaking in cloves and rosemary extracts to significantly decrease acrylamide in chips. Besides NaCl, other soaking agents also were used to improve sensorial quality of potato chips, e.g. citric acid or combination of NaCl and citric acid (Basuny and

Arafat, 2013; Abou - Zaid, 2015; Calder *et al*., 2011; Tsouvaltzis and Brecht, 2016) and solution of $CaCl_2$ (Khalaf *et al*., 2015). Soaking before frying caused considerably better evaluated color in French fries (Burch *et al*., 2008; Ismial *et al*., 2013).

Drying pretreatment also can show the positive effects on acrylamide and fat reduction in chips and fries (Pedreschi *et al*., 2007; Kampuse *et al*., 2014). Abdula *et al*. (2013) reported that pre-drying method in combination with soaking in citric acid solution improved sensorial properties of sweet potato chips.

The aim of this study was to investigate the effect of pretreatment by soaking in salty solutions of different powdered agents (sweet paprika, ginger and curry mixture) and additional drying pretreatment on quality of fried potato chips. The major task of this study was to produce quality slightly aromatized potato chips by dissolved compounds from soaking solutions. Soaking and drying pretreatments were performed with the purpose to improve the chemical and sensorial properties of fried chips. That quality improvement would be recognized in changes of chemical (moisture and fat content decreasing) and sensorial properties (better color, crispness (texture), taste and overall acceptability) of soaked samples in comparison with non-soaked blanched sample. Drying pretreatment was used to additionally improve chips quality by higher moisture loss and betterment of texture/crispness and overall acceptability.

MATERIALS AND METHODS

Material

Potatoes (*Solanum tuberosum*) unknown variety, frying oil (Zvijezda, Croatia), soaking agents: table salt (Solana, Tuzla), powdered curry (Gameha, Sarajevo), powdered ginger (Gameha, Sarajevo), powdered sweet paprika (Franck, Croatia) were collected from local market in Sarajevo.

Methods of preparation

Potatoes were washed in tap water, peeled, sliced to the thickness between 1.5 and 1.8 mm and rinsed in distilled water to eliminate starch from surface. Total number of samples was seven (six soaked and one blanched). The soaking solutions were prepared using 200 mL of distilled water (in six batches) and 2g of NaCl (1%) and 1 g of each soaking agent (1%). Prepared soaking solutions with dissolved NaCl and soaking agents were heated to 60°C and potato slices were added in (about 80 g of potato in 200 mL of solution.). Soaking lasted 60 minutes. After soaking a part of each soaked sample was drained and fried immediately at 170°C for 5 minutes, and another part was drained and dried at 50°C for 30 minutes and fried at 170°C for 3 minutes. One sample was blanched in water at 70°C for 5 minutes without soaking pretreatment and fried. Samples were fried in kitchen fryer (Neo, Type: N DF 10, capacity 1 L). Potato/oil ratio during frying was 80 g of raw potato / 1 liter of oil. The list of samples with pretreatment methodology is given in Tab. 1.

Analysis

Chemical analysis included: determination of: 1) fat by Soxshlet extraction during 6 hours using diethyl ether as solvent, 2) moisture gravimetrically by drying in laboratory oven at 105°C for 2.5 hours (AOAC, 2000) and 3) NaCl by Mohr titration (E.K. 8045, JUS., 1993).

Sensorial evaluation included the assessment of following sensorial attributes: color, texture/crispness, odor, taste and overall acceptability. Semi trained panelists (n=12) were selected for sensory analysis. Each attribute was evaluated using a 5-Point hedonic scale (5- excellent - extremely acceptable, 4-good – acceptable, 3- neither like nor dislike 4–dislike slightly but satisfy, 1-dislike extremely, extremely unacceptable).

All determinations were carried out in triplicate and data were reported as mean ± standard deviation. Significant influence of pretreatment was tested using one-way ANOVA, and significant differences ($p<0.05$) were calculated using Tukey test.

Table 1 Experimental design - list of samples and preparation methodology
Tabela 1 Dizajn eksperimenta – popis uzoraka i opis metodologije

Potato chips samples / *Uzorci čipsa od krompira*	Preparation methodology / *Metode pripreme*		
	Composition of soaking solution, soaking conditions / *Sastav rastvora za uranjanje, uslovi uranjanja*	Drying conditions / *Uslovi sušenja*	Frying conditions / *Uslovi prženja*
Ginger SC	1% NaCl + 1% powdered sweet paprika / *1% NaCl + 1% mljevena slatka paprika,* 60°C, 60 min	-	170°C, 5 min
Ginger SC-D	1% NaCl + 1% powdered sweet paprika / *1% NaCl + 1% mljevena slatka paprika,* 60°C, 60 min	50°C, 30 min	170°C, 3 min
Paprika SC	1% NaCl + 1% powdered ginger / *1% NaCl + 1% đumbir u prahu,* 60°C, 60 min	-	170°C, 5 min
Paprika SC-D	1% NaCl + 1% powdered ginger / *1%NaCl + 1% đumbir u prahu* 60°C, 60 min	50°C, 30 min	170°C, 3 min
Curry SC	1% NaCl + 1% powdered curry / *1%NaCl + 1% kari u prahu* 60 °C, 60 min	-	170°C, 5 min
Curry SC-D	1% NaCl + 1% powdered curry / *1%NaCl + 1% kari u prahu* 60°C, 60 min	50°C, 30 min	170°C, 3 min
Blanched chips	-	-	170°C, 5 min
	Blanched in water at 70°C for 5 minutes *Blanširan u vodi na 70℃ 5 minuta*		

SC – soaked chips samples, SC-D – soaked and dried chips samples /
SC – uzorci čipsa uranjani u rastvore prije prženja, SC-D – uzorci čipsa uranjani i sušeni prije prženja

RESULTS AND DISCUSSION

Chemical analysis

Significantly highest level of moisture content was noticed in blanched chips (Tab. 2). High moisture content is undesirable in potato chips as crispy product. Moisture decreases crispness of potato chips. All samples pretreated by drying before frying had lower moisture than samples fried without drying. Because of that those samples had better crispness (Tab 3). Fat content was pretty high in all samples (36.86-47.40%) and pretreatments did not decrease fat level. The lowest fat content was noticed in blanched chips. The fat content in blanched sample was significantly lower than in samples pretreated in ginger and paprika solution without drying. Results of NaCl content were expected. The salt content was significantly higher in samples that were dried before frying. The lowest significant NaCl level was noticed in blanched sample.

Table 2 Results of chemical analysis of pretreated potato chips
Tabela 2 Rezultati hemijskih analiza predtretiranih uzoraka čipsa

Sample / *Uzorak*	Moisture / *Vlaga* (%)	Fat / *Mast* (%)	NaCl (%)
Ginger SC	2,75±0,23[b]	46,51±0,71[a]	1,94±0,07[d]
Ginger SC-D	2,70±0,16[b]	41,73±0,94[ab]	2,24±0,18[c]
Paprika SC	3,05±0,40[b]	44,18±1,39[ab]	2,15±0,08[cd]
Paprika SC-D	2,33±0,20[b]	47,40±1,94[a]	2,65±0,06[b]
Curry SC	3,20±0,38[b]	43,19±0,13[ab]	2,56±0,02[b]
Curry SC-D	2,57±0,25[b]	45,82±5,08[ab]	3,09±0,05[a]
Blanched chips	4,77±0,52[a]	36,86±2,54[b]	0,00±0,00[e]

Different small letters in columns represent significant differences between samples
Različita mala slova u kolonama označavaju statistički značajne razlike između označenih uzoraka
SC – soaked chips samples, SC-D – soaked and dried chips samples /
SC – uzorci čipsa uranjani u rastvore prije prženja, SC-D – uzorci čipsa uranjani i sušeni prije prženja

The results for chemical analysis are in accordance with literature data (Khalaf *et al.*, 2015; Tahmaz and Smajić, 2006; Tahmaz, 2005: Souci *et al.*, 1990). It is known that soaking in 1% citric acid solution can decrease fat content for about 7% comparing to control Khalaf *et al.* (2015). According to the fat content in industrial potato chips was in the range 33.26 – 39.86%. Moisture content in industrial produced chips is usually in the range 0.89 -2.95% (Tahmaz and Smajić, 2006). According to Khalaf *et al.*, (2015) potato chips soaked in citric acid solution contained 2.85% of moisture (Khalaf *et al.*, 2015). The usual content of salt in industrial potato chips is in the range 0.57-2.40% (Tahmaz and Smajić, 2006; Soucci *et al.*, 1999).

Sensorial evaluation

Blanched sample had the worst evaluated sensorial characteristics (Tab. 3). The scores for overall acceptability (3.25) and taste (2.83) for this sample were the lowest in comparison with other samples. The best sensorial acceptability was noticed in curry soaked sample which was dried before frying. This sample had the highest scores for texture, odor, taste and overall acceptability. Pretty good sensorial properties were noticed in sample soaked in ginger solution, also with additional drying.

Table 3 Results of sensorial evaluation of pretreated potato chips
Tabela 3 Rezultati senzorne ocjene predtretiranih uzoraka čipsa

Samples / *Uzorci*	Color / *Boja*	Texture and crispness / *Tekstura i hrskavost*	Odor / *Miris*	Taste / *Okus*	Overall acceptability / *Opšta prihvatljivost*
Ginger SC	4.42±0,79^{ab}	4.33±0,65^{ab}	3.67±0,49^{ab}	3.42±0,79^{bc}	4.00±0,60^{abc}
Ginger SC-D	4.83±0,39^{a}	4.50±0,52^{a}	4.00±0,85^{ab}	4.25±0,42^{ab}	4.42±0,51^{a}
Paprika SC	3.58±0,51^{c}	4.17±0,83^{ab}	3.67±0,65^{ab}	3.58±0,79^{bc}	3.58±0,51^{bc}
Paprika SC-D	3.83±0,72^{bc}	4.42±0,67^{ab}	4.08±1,00^{ab}	4.25±0,87^{ab}	4.08±0,79^{ab}
Curry SC	4,00±0,74^{abc}	4,42±0,67^{ab}	3,92±0,51^{ab}	4,00±0,85^{ab}	4,17±0,58^{ab}
Curry SC-D	4,00±1,04^{abc}	4,58±0,69^{a}	4,17±0,94^{a}	4,67±0,65^{a}	4,50±0,80^{a}
Blanched	3.67±0,49^{bc}	3.75±0,62^{b}	3.17±0,58^{b}	2.83±0,58^{c}	3.25±0,45^{c}

Different small letters in columns represent significant differences between samples
Različita mala slova u kolonama označavaju statistički značajne razlike između označenih uzoraka
SC – soaked chips samples, SC-D – soaked and dried chips samples /
SC – uzorci čipsa uranjani u rastvore prije prženja, SC-D – uzorci čipsa uranjani i sušeni prije prženja

Color sensory evaluation of pretreated samples varied from 3.58 to 4.83. Differences in color between samples are showed in Fig. 1 and 2. The best evaluated color had sample soaked in ginger solution with additional drying pretreatment. This sample had the lightest color (Fig. 2). The darkest chips samples had the lowest evaluation for color (sample soaked in paprika solution without additional drying – 3.58 and blanched sample – 3.67). Samples pretreated in ginger solution had the highest scores for color, probably because of the brightest color and similarity with industrial chips. Although the color of curry pretreated chips was partly unspecific, these samples were evaluated with high scores (4.00 - acceptable/good) in both cases (dried and non-dried). This bright intensive yellow color is unspecific for potato chips, because the industrial chips color is different (different hue of yellow color). On the other hand, samples pretreated in paprika solution had the worst evaluation for color, because of dark color of chips especially in sample without additional drying (Fig. 1).

Figure 1 Chips samples obtained without additional drying pretreatment
Slika 1 Uzorci čipsa dobiveni bez dodatnog predtretmana sušenjem

Figure 2 Potato chips samples obtained by combination of soaking and drying pretreatments
Slika 2 Uzorci čipsa dobiveni kombinacijom predtretmana uranjanjem i sušenjem

The affinity of paprika soaked samples to be over-fried and too much dark can be explained by presence of carotenoids (dark red color) in paprika and higher concentration of reduced sugars then in ginger and curcuma (Moreschi *et al*., 2006; Zaki *et al*., 2013; Ajaykumar *et al*., 2012; Sharma *et al*., 2015). Additionally, longer exposure to high frying temperature (170° - 5 minutes) could explain darker color of non-dried chips samples in comparison with samples pretreated by drying (fried 3 minutes at 170°C). It is known that heating over 100°C causes Millards reaction and darkening. Darkening is more intensive if temperature is higher.

The samples soaked in curry solutions had very interesting intensive yellow color (Fig. 1 and 2), pleasant odor, aroma and taste. These samples were slightly aromatized with curry spices. There are no literature data about application of curry mixture in technology of potato chips. All sensorial characteristics of curry chips samples were evaluated as good (acceptable) with slightly pretension to become excellent (from 3.92 to 4.58). It is well known that curcuma from curry mixture has good antioxidant properties (Rachana and Venugopalan, 2014). This fact gives possible additional value of this chips type, which should be more studied. It is supposed that curcuma applied in potato chips preparation could avoid oxidative degradation of oil, decrease acrylamide formation and improve anti-oxidative activity of fried chips and made it as healthier product. Similar as curcuma, active compounds from ginger root (tannins, polyphenols, flavonoids, carotenoids, ascorbic acid) and paprika (carotenoids, capsicum, ascorbic acid) also poses considerable anti-oxidative activity (Shirin Adel and Prakash, 2010; Nadeem *et al*., 2011).

Taste, texture, odor and overall acceptability were better in soaked samples than in blanched. All soaked samples were slightly aromatized by soaking agents, which resulted to better taste and odor, especially in dried samples. It can be seen that application of curry gave the best results in taste and overall acceptability evaluation. Soaking in ginger with additional drying also made good results, especially for color of fried chips (Fig. 1, Tab. 2). It is very important to notice that there are no samples which were evaluated as unacceptable. For overall acceptability samples were mostly evaluated as acceptable/good (~4.00). The scores varied from 3.25 (blanched - neither like nor dislike) to 4.56 (soaked in curry and dried - acceptable to extremely acceptable). Chips soaked in paprika solution had somewhat lower scores, but still acceptable. The main reason of lower scores for paprika soaked samples could be related to darker color and slightly bitter taste.

The results are mostly in agreement with data reported by other authors. Gaikwad and Athmaselvi (2016), Abou – Zaid (2015), Ismial *et al*. (2013) and Bunger *et al*. (2003) reported that potato chips and French fries had improved color, texture, taste and overall acceptability when were soaked in NaCl solution in comparison with blanched non-soaked samples. Abou – Zaid (2015) reported that potato chips samples pretreated by soaking in solution prepared with NaCl, citric acid and tomato juice had improved sensorial characteristics. According to Ismial *et al*. (2013) that samples soaked in NaCl for 60 minutes had the best sensorial properties, the lightest color, improved taste and overall acceptability. The worst taste, texture and overall acceptability had blanched sample, which was in agreement with our results and observations (Tab. 3, Fig. 1 and 2)

The studies about application of natural antioxidant sources from plant extracts like clove and rosemary (El – Shawaf *et al*., 2014) or green tea extract and lycopene (Aydeniz and Yilmaz, 2016) showed similar results for sensorial properties. According to El – Shawaf *et al*. (2014) plant extracts pretreated chips samples had improved color, texture and overall acceptability.

It can be seen from Tab. 2 and Fig. 1 and 2 that all dried samples had better sensorial characteristics in comparison with non-dried samples soaked in same solution. This fact would be explained by lower moisture content in dried samples. Mainly because of the reason when moisture level decreased, the crispness increased. When each soaking pretreatments with and without additional drying process is compared, it can be seen that all dried samples also had significantly ($p \leq 0.05$) higher NaCl content for all soaking solutions. This considerable higher NaCl content could be explained by greater moisture loss in dried samples and consequently higher concentration of total dry mater. On the other hand, drying pretreatment did not manage to decrease fat uptake with only exception of ginger soaked samples. But these differences in fat were not significant. Generally, all sensorial properties were found as better in dried samples (Tab.3). In try to explain this, it could be supposed that higher moisture loss probably caused better acceptability of all sensorial attributes. It is well known that crispness increases when moisture decreases and because of that the texture was better. For the same reason, the aroma and taste compounds probably were better concentrated in samples with higher moisture loss, which finally resulted in better taste and odor.

CONCLUSION

Soaking of potato slices in different solutions before frying had influence on improvement of chips quality. The best sensorial acceptability was noticed in samples pretreated in curry and ginger solutions.

Samples soaked in curry solution had amazing good sensorial characteristics and were evaluated as good (4.17 without drying) and good to excellent (4.50 – with drying) because of specific taste and intensive yellow color. These samples had the best taste, odor and overall acceptability. The suggestion for future is to research different concentrations of NaCl and curry in soaking solution and for their possible application in industrial production.

The lightest and most acceptable color was noticed in ginger pretreated chips. This soaking agent could be more researched for future industrial application.

Generally the worst sensorial properties were noticed in blanched (not salty enough, dark color) sample and in samples soaked in paprika solution (over-fried, dark color and bitter taste). Because of that application of paprika as soaking agent could not be suggested for future practical application.

All soaked samples had better texture than blanched. Neither sample was evaluated as unacceptable.

In the term of chemical characteristic, all pretreated samples had significantly ($p \leq 0.05$) lower moisture content, which resulted in better crispness and overall acceptability. Unlike moisture, the fat content did not decrease in pretreated soaked samples.

In comparison with blanched chips, it can be seen that soaking pretreatment had significant ($p \leq 0.05$) influence on moisture decreasing, NaCl increasing and mostly (not significant in all cases) improvement of sensorial properties.

Drying after soaking caused better moisture loss, better crispness and overall acceptability in all soaked samples.

REFERENCES

A.O.A.C. (2000): Association of Official Method of Analysis. Official Analytical Chemists. 17th Ed., Gaithersburg, MD: AOAC International.

Abdulla, G., El-Shourbagi, A. G., Sitohi, M. Z. (2014): Effect of Pre-drying, Blanching and Citric Acid Treatments on the Quality of Fried Sweet Potato Chips, American Journal of Food Technology, 9, (1): 39-48.

Abou-Zaid, F. O. F. (2015): The effect of using some treatments on reduction of acrylamide formation in processed potatoes, Agri-Industrialization Unit, Plant Production Department, Desert Reasearch Center, Cairo, Egypt

Ajaykumar, T. M., Sandeep, J. L., Mandhukar, B. G. (2012): Effect of Pretreatments on Quality Attributes of Dried Green Chili Powder, ISCA Journal of Engineering Sciences, Vol. 1(1), 71-74,

Aydeniz, B., Yilmaz, E. (2016): Performance of different natural antioxidant compounds in frying oil. Food Technology & Biotechnology 54 (1): 21-30.

Basuny, A. M. M., Arafat, S. M. (2013): Reduction of acrylamide formation, polymer compounds and oil uptake in potato slices during frying. Current Research in Microbiology and Biotechnology, Vol. 1, No. 3 (2013): 111-118

Bunger, A., Moyano, P., Rioseco, V. (2003): NaCl soaking treatment for improving the quality of French-fried potatoes. Food Res Int 36:161–166.

E.K. 8045, JUS. (1993): Određivanje sadržaja natrijum-hlorida, Službeni list SRJ 27/93

El-Shawaf, M. G, El-Zamzamy, F. M., Mekky, T. M. (2014): Reduction of Acrylamide Formation in Potato Chips Using Natural Antioxidants Sources from Plant Extracts, Middle East Journal of Agriculture Research, 3(1): 89-99.

Fellows, P. (2000): Frying, In: Food processing technology, Principles and practices, 2nd edition, CRC Press, Boca Raton, Boston, New York, Washington, 355-362.

Gaikwad, K. Y., Athmaselvi, K. A. (2016): Effect of soaking pre-treatments on different characteristics of fried potato chips, Department of Food Process Engineering, School of Bioengineering, Food Safety and Quality Management, SRM University, Chennai, India

Ismial, S. A-M. A., Ali, R. F. M., Samy, W. M. (2013): Impact of pre-treatments on the acrylamide formation and organoleptic evolution of fried potato chips, American Journal of Biochemistry and Biotechnology, 9 (2): 90-101.

Kampuse, S., Jefimovs, A., Rakcejeva, T. (2014): The influence of pre-treatment method on the fat content decrease in French fries, Department of Food Technology, Faculty of Food Technology, Latvia University of Agriculture, Jelgava, Latvia

Khalaf, H. H. A., Sharoba, A. M., El-Desouky, A. I., El-Bassiony, K. R. A., Afifi, S. A. M. (2015): Effect of some pre-treatments on acrylamide concentration in potato chips, Annals of Agric. Sci. Vol. 53(2), ISSN 1110-0419) 211–220.

Mehta, U., Swinburn, B. (2001): A review of factors affecting fat absorption in hot chips. Critical Reviews in Food Science and Nutrition, No.41, p.133–154.

Mestdagh, F., De Wilde, T., Fraselle, S., Govaert, Y., Ooghe, W. Degroodt, J. M., Verhe R., Van Peteghem, C. and De Meulenear, B. (2008): Optimization of the blanching process to reduce acrylamide potatoes. LWT-Food Science and Technology, 41(9)

Moreschi, S. R. M., Leal, J. C., Braga, M. E. M., Meireles, M. A. A. (2006): Ginger and turmeric starches hydrolysis using subcritical water+CO2: The effects of the SFE pretreatments. Brazilian Journal of Chemical Engineering Vol. 23, No. 02, pp. 235 – 242.

Moreira, R. G. (2007): Deep fat frying, Heat transfer in food processing. WIT Transactions on State of the Art in Science and Engineering, Vol. 13, © 2007 WIT Press, 209-236.

Nadeem, M., Anjum, F. M., Khan, M. R., Saeed, M., Riaz, A. (2011): Antioxidant Potential of Bell Pepper (*Capsicum annum* L.)-A Review. Pakistan Journal of Food Sciences, Vol. 21, Issue 1-4, 45-51.

Onishi, Y., Prihanto, A. A., Yano, S. Takagi, K., Umekawa, M., Wakayama, M. (2015): Effective treatment for suppression of acrylamide formation in fried potato chips using L-asparaginase from *Bacillus subtilis*, 3 Biotech 5:783–789.

Pedreschi, F., Leo´n, J., Mery, D., Moyano, P., Pedreschi, R., Kaack, K. Granby, K. (2007): Color development and acrylamide content of pre-dried potato chips, Journal of Food Engineering 79 (2007) 786–793.

Rachana, S., Venugopalan, P. (2014): Antioxidant and bactericidal activity of wild turmeric extracts. Journal of Pharmacognosy and Phytochemistry 2 (6): 89-94.

Sharma, R., Joshi, VK, Kaushal, M. (2015): Effect of pre-treatments and drying methods on quality attributes of sweet bell-pepper (*Capsicum annum*) powder. J Food Sci Technol (June 2015) 52(6):3433–3439.

Shirin Adel, P. R., Prakash, J. (2010): Chemical composition and antioxidant properties of ginger root (*Zingiber officinale*). Journal of Medicinal Plants Research Vol. 4(24), pp. 2674-2679

Souci, S. W., Fachmann, W., Kraut, H. (1990): Food composition and nutrition tables, Wissenschaftliche verlagsgesellschaft mbH, Sttutgart.

Tahmaz, J. (2005): Proizvodnja i kavalitet čipsa i srodnih proizvoda, Magistarski rad, Poljoprivredni fakultet Univerziteta u Sarajevu, Sarajevo.

Tahmaz, J., Smajić, A. (2006): Extruding proces characteristics and differences between extruded and non-extruded potato snacks, Works of Faculty of Agriculture-University of Sarajevo, No.58/2.

Tsouvaltzis, P., Brecht, JK. (2016): Inhibition of Enzymatic Browning of Fresh-Cut Potato by Immersion in Citric Acid is Not Solely Due to pH Reduction of the Solution, Journal of food processing and preservation 9 (2): 90-101.

Zaki, N., Hakmaoui, A., Ouatmane, A., Fernandez-Trujillo, J. P. (2013): Quality characteristics of Moroccan sweet paprika (*Capsicum annuum* L.) at different sampling times. Food Sci. Technol, Campinas, 33(3): 577-585.

DISTRIBUTION OF VOLATILE SULFUR COMPOUNDS AND HEAVY METALS IN KOHLRABI AND LEEK*

Mario Nikola Mužek[1]**, Azra Đulović[1], Maša Buljac[1], Dario Omanović[2], Tomislav Kozina[1], Andrea Sedlar[1], Lucija Radaš[1], Sandra Svilović[1], Ivica Blažević[1]

Original scientific paper

Summary

Volatile sulfur compounds and heavy metals were investigated in different parts of the two plants: kohlrabi (*Brassica rupestris* Raf. ssp. *gongyloides* (L.) Janch.; Brassicaceae family) and leek (*Allium porrum* L.; Amaryllidaceae family). Owing to the presence of sulphur, volatile sulphur compounds show particular physicochemical features and interact with a range of molecular and biological targets. The main volatiles in *B. rupestris* were found to be degradation products of glucosinolates: sinigrin (allyl isothiocyanate), glucotropaeolin (benzeneacetonitrile and benzyl isothiocyanate), gluconasturtiin (2-phenylethyl isothiocyanate), and glucoreucin (4-(methylsulfanyl)butyl isothiocyanate), as well as from degradation of *S*-methylcysteine sulfoxide commonly named methiin (dimethyl trisulfide and dimethyl tetrasulfide). The main volatiles in *A. porrum* were derived from *S*-cysteine sulfoxide degradation: methiin, propiin, and isoaliin (methyl propyl trisulfide, (*E*)-propenyl propyl trisulfide, dipropyl disulfide, (*E*)-propenyl propyl disulfide, dimethyl trisulfide, dipropyl trisulfide). Concentrations of various heavy metals have also been determined. The concentrations of heavy metals determined were in sequence Al > Fe > Mn > Zn > Cu > Ni > Pb > Mo > As > Co > Cd > Se for kohlrabi and Al > Fe > Mn > Zn > Cu > Ni > Mo > Pb > Co > Cd > As, Se for leek, respectively.

Keywords: *Brassica rupestris, Allium porrum, glucosinolates, S-cysteine sulfoxides, heavy metals*

INTRODUCTION

Volatile sulphur compounds make up a large group of different species found in a 43 plant families and 173 plant genera, including the most important families, Brassicaceae, Amaryllidaceae, Capparaceae, Caricaceae, Rutaceae, Phytolaccaceae and Poaceae (Iranshahi, 2012). Glucosinolates, β-thioglucoside-*N*-hydroxysulfates

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] University of Split, Faculty of Chemistry and Technology
[2] Ruđer Bošković Institute, Zagreb
**Corresponding author: muky@ktf-split.hr

with variable side chains are secondary metabolites identified in over 16 different plant families (Blažević *et al*., 2016). These compounds are found particularly in the Brassicaceae family which encompasses *ca.* 330 genera and over 3700 species among which *Brassica* genus stands out. It includes *ca*. 38 species among which many are important agricultural and horticultural crops such as *Brassica oleracea* (broccoli, cabbage, cauliflower, kale, etc.), *Brassica rapa* (turnip, Chinese cabbage, etc.), *Brassica napus* (rapeseed, etc.). *Brassica* vegetables are highly regarded for their nutritional value. They provide high amounts of vitamin C and soluble fibres and contain nutrients with anticancer properties such as 3,3'-diindolylmethane, sulphoraphane (glucosinolate degradation products), and selenium (Finley *et al*., 2005; Banerjee *et al*., 2012).

In the APG IV classification system, *Allium* is placed in the family Amaryllidaceae. In some of the older classification systems, *Allium* was placed in Liliaceae (Chase *et al*., 2009). *Allium* is one of about 57 genera of flowering plants with more than 500 species and represents by far the largest genus in the Amaryllidaceae family. Plants of the *Allium* genus produce *S*-alk(en)yl-L-cysteine-sulfoxides — odorless, characteristic non-volatile sulphur secondary metabolites, which, following subsequent hydrolysis, give rise to many volatile compounds, including thiosulphinates and (poly)sulphides responsible for specific *Allium* flavour and health promoting effects. Many are used as food plants because in most cases, both bulb and leaves are edible.

Toxic metals and metalloids are important environmental pollutants, and many of them are toxic even at very low concentrations. Plants evolved the ability to take up, tolerate and accumulate exceptionally high concentrations of metals and metalloids present in the soil (and water) and, more importantly, in their aboveground biomass without visible toxicity symptoms. The term "hyperaccumulator" refers for plants that actively take up exceedingly large amounts of one or more heavy metals from the soil without showing symptoms of phytotoxicity. Heavy metals are not retained in the roots but are translocated to the shoot and accumulated in the aerial parts, especially leaves, at concentrations 100–1000-fold higher than those found in non-hyperaccumulating species (Rascio and Navari – Izzo, 2011). Considering the molecular mechanisms used in achieving its resistance/tolerance towards metal stress, i.e. reduction of negative impact of metal toxicity, plant species can be viewed as: heavy metal-sensitive plants (plants that cannot keep metals out of their roots or prevent transport to the shoot, and which will succumb due to the toxic effects of metals on root and shoot cells), heavy metal-resistant excluder plants (plants able to keep metals outside the roots or take care of rapid efflux in case toxic metals have entered root cells), heavy metal-tolerant non-hyperaccumulator plants (metals can enter root cells where they are sequestrated into root vacuoles, preventing translocation to shoots), and heavy metal-hypertolerant hyperaccumulator plants (metals are actively taken up through the root and largely loaded into xylem for root to shoot transport and afterwards the metals are safely sequestered in vacuoles) (Lin and Aarts, 2012). To date 34 different families (Asteraceace, Brassicaceae, Caryophyllaceae, Poaceae, Violaceae and Fabaceae) including 90 species from 11

genera have been reported for their trace metal-tolerance and/or accumulation capacity. In relation to metal hyperaccumulation, species of the Brassicaceae family represent the largest family that include species that possess this ability. Some of the representative plant species extensively investigated for metal hyperaccumulation include *Brassica* sp., *Alyssum* sp., *Arabidopsis* sp. and *Thlaspi* sp. Among those plants, *Brassica juncea*, and *Thlaspi caerulescens* served as the model plants for heavy metal-tolerant non-hyperaccumulator and metal-hypertolerant hyperaccumulator studies. Four species in particular, *Alyssym* sp., *Thlaspi caerulescens*, *Thlaspi rotundifolium* and *Arabidopsis halleri* have been studied extensively for their ability to hyperaccumulate several trace metals, including Zn, Cd and Ni. Moreover, the majority of the metal hyperaccumulators from the family Brassicaceae are Ni hyperaccumulators (Anjum *et al*., 2012). The only plant from the family Amaryllidaceae reported as metal-hyperaccumulator is *Allium ampeloprasum* for Cr, Cu, and Pb, as heavy metals (Ansari *et al*., 2015).
Soil pH is a measure of the acidity or basicity of a soil and is considered a master variable in soils as it affects many chemical processes. It specifically affects plant nutrient availability by controlling the chemical forms of the different nutrients and influencing the chemical reactions they undergo. The optimum pH range for most plants is between 5.5 and 7.5; however, many plants have adapted to thrive at pH values outside this range. According to the pH value, soil can be categorised as: very strongly acidic (pH < 4), strongly acidic (pH = 4 - 5), moderately acidic (pH = 5 - 6), slightly acidic or neutral (pH = 6 - 7), neutral or slightly alkaline (pH = 7 - 8), moderately alkaline (pH = 8 - 9), strongly alkaline (pH = 9 - 10), very strongly alkaline (pH > 10) (Ilijanić and Gračanin, 1977).
The aim of the work was to uncover sulfur containing constituents by isolation of the volatiles using hydrodistillation in Clevenger type apparatus followed by GC-MS analyses and to determine concentrations of various heavy metals using HR ICP-MS in different parts of the two plants: *Brassica rupestris* Raf. ssp. *gongyloides* L. (kohlrabi) and *Allium porrum* L. (leek).

MATERIALS AND METHODS

General

All the solvents employed were purchased from Fluka Chemie, Buchs, Switzerland. Anh. Na_2SO_4, KCl, HNO_3 and HCl were obtained from Kemika, Zagreb, Croatia. For volatile sample concentration Dri-Block heater (Techne, UK) was used. Gas chromatography analyses were performed on gas chromatograph (model 3900; Varian Inc., Lake Forest, CA, USA) equipped with mass spectrometer (model 2100T; Varian Inc.), and non-polar capillary column VF-5MS (30 m × 0.25 mm i.d., coating thickness 0.25 μm). The pH value of soil was measured by Mettler Toledo SevenEasy instrument. Heavy metals analysis was conducted by High Resolution Inductively Coupled Plasma Mass Spectrometer (HR ICP-MS) Element 2, Thermo Finnigan, Bremen, Germany.

Plant material

Brassica rupestris Raf. ssp. *gongyloides* L. (kohlrabi) and *Allium porrum* L. (leek) were collected in April 2017. from cultivated plants in Gata (near Omiš), Croatia. The kohlrabi and leek were divided into different parts for analysis: the underground part and the above ground portions. Kohlrabi was divided into root (underground part) and bulb, stem, leaves and flowers (above ground parts). The leek was divided into root and bulb (underground parts) and leaves (above ground part).

Isolation of Volatiles

Dried aerial parts of plant material (100 g) were subjected to hydrodistillation for 3 h in a Clevenger type apparatus. The obtained volatile fraction was dried over anhydrous sodium sulphate. The volatile samples were concentrated using nitrogen stream at 40 °C and in Dri-Block.

GC/MS Analysis

VF-5MS column temperature was programmed at 60 °C isothermal for 3 min, and then increased to 246 °C at a rate of 3 $°C \cdot min^{-1}$ and held isothermal for 25 min. Other chromatographic conditions were: carrier gas helium; flow rate 1 $mL \cdot min^{-1}$; injector temperature 250 °C; volume injected 1 μL; split ratio 1:20; ionization voltage 70 eV; ion source temperature 200 °C; mass scan range: 40 - 350 mass units.

Identification and Quantification of Components

The individual peaks were identified by comparison of their retention indices (relative to C8 - C40 n-alkanes for VF-5MS) to those from a homemade library, literature and/or authentic samples, as well as by comparing their mass spectra with literature, Wiley 7 MS (Wiley, New York, NY, USA) and NIST02 (Gaithersburg, MD, USA) mass spectral databases. The homemade library was created from authentic compounds obtained commercially and from the main components of many essential oils obtained during our previous studies.

Soil pH determination

Soil sample (10 g) is placed in a 50 ml baker, a 25 ml volume of distilled water is added and soil is stirred for 30 min. Then the pH is measured with the glass electrode.
According to the pH values obtained, the soil is classified into one of the groups (Ilijanić and Gračanin, 1977).

Heavy metals analysis

Plant materials were prepared for heavy metals analysis by dry ashing method. Dried, ground plant part (1 g) was weight in a porcelain crucible and placed in a cool muffle furnace and ashed at 500 °C overnight. After cooling, the ash was dissolved in 5 mL of 20% HCl. The solution was filtered through an acid-washed filter paper into a 50 mL volumetric flask. The filter paper was washed and the solution was diluted to volume with deionized water and mixed well. HR ICP-MS was used for the determination of dissolved element concentrations in prepared solutions. The samples for the analyses were prepared in pre-cleaned polyethylene tubes by adding 100 µL of concentrated HNO_3 and 50 µL of indium(115) internal standard (0.1 mg L^{-1}) into 5 mL of a sample aliquot. The concentrations of the elements were determined by means of external calibration plots. No special setup of the instrument operating conditions was needed. Quality control (QC) of HR ICP-MS measurements was checked by the determination of elements concentration in "River Water Reference Material for Trace Metals" (SLRS-5, National Research Council Canada). For most elements, a good agreement with the certified data was obtained.

RESULTS AND DISCUSSION

Volatile sulfur compounds in kohlrabi and leek

Tables 1. and 2. represent the results of the GC-MS analysis from different plant parts of kohlrabi and leek as well as their non-volatile precursors.

Table 1. Identified glucosinolate and *S*-alk(en)yl cysteine sulfoxide degradation products in different plant parts of *Brassica rupestris* Raf. ssp. *gonglyoides*

Brassica rupestris Raf. ssp. *gonglyoides*			
Plant parts	**Glucosinolate or *S*-alk(en)yl cysteine sulfoxide /** Identified degradation products	**Retention time (min)**	**Percentage (%)**
Bulb	**Sinigrin**		
	Allyl isothiocyanate	6.00	0.28
	Glucotropaeolin		
	Benzenacetonitrile	17.30	0.16
	Glucolimnanthin		
	2-Methoxyphenyl acetonitrile	22.90	0.25
	Gluconasturtiin		
	Benzenepropanenitrile	21.94	0.25
	2-Phenylethyl isothiocyanate	31.89	0.32
	Methiin		
	Dimethyl trisulfide	8.87	4.24
	Methyl(methylthio)methyl disulfide	16.24	0.10
	Dimethyl tetrasulfide	20.30	0.16
Root	**Sinigrin**		
	Allyl isothiocyanate	6.00	11.50
	Glucotropaeolin		
	Benzenacetonitrile	17.30	0.86
	Glucoerucin		
	4-(Methylsulfanyl)butyl isothiocyanate	30.50	1.76
	Gluconasturtiin		
	Benzenepropanenitrile	21.94	7.07
	2-Phenylethyl isothiocyanate	31.89	1.64

	Methiin Dimethyl trisulfide Methyl(methylthio)methyl disulfide Dimethyl tetrasulfide	 8.87 16.24 20.30	 7.92 0.38 1.41
Stem and leaf	**Sinigrin** Allyl isothiocyanate	 6.00	 0.44
	Glucotropaeolin Benzenacetonitrile	 17.30	 0.13
	Methiin Dimethyl trisulfide Methyl(methylthio)methyl disulfide Dimethyl tetrasulfide	 8.87 16.24 20.30	 4.26 0.06 0.26
Flower	**Sinigrin** Allyl isothiocyanate	 6.00	 2.45
	Glucotropaeolin Benzenacetonitrile	 17.30	 0.32
	Methiin Dimethyl trisulfide Methyl(methylthio)methyl disulfide Dimethyl tetrasulfide	 8.87 16.24 20.30	 15.46 0.02 0.16

The obtained results showed that the sulfur volatiles originated from the present glucosinolates which represent chemical tags of the family, but also from cystein sulfoxide degradation. Glucosinolates can degrade enzymatically, chemically, and thermally (Blazevic *et al*., 2014). Distillation in Clevenger at 100°C insures thermal degradation and isolation with the main volatile isothiocyanates and nitriles.

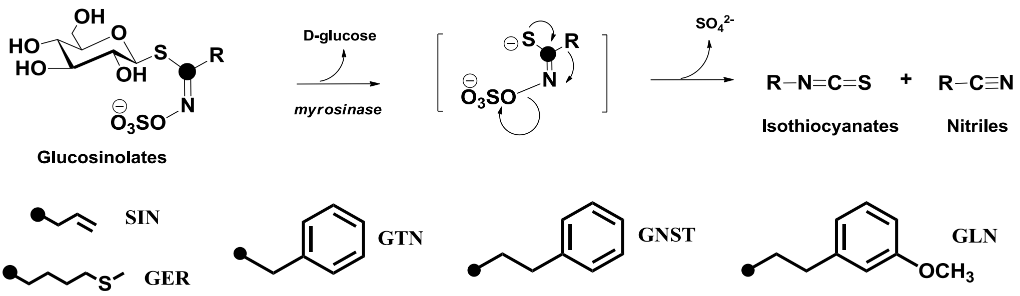


Figure 1. General scheme of glucosinolate degradation with the structures identified in the investigated plants.

Allyl isothiocyanate was identified in all volatile fractions in the following order root, flower, stem and leaf, and bulb having 11.5%, 2.5%, 0.44%, and 0.28%, respectively. It originates from degradation of the allylglucosinolate known as sinigrin. Other aliphatic glucosinolate degradation product was 4-(methylsulfanyl)butyl isothiocyanate (erucin), found in root (1.76%), that originates from glucoerucin. Three aromatic glucosinolates were also detected based on their volatiles:

benzeneacetonitrile was found in all investigated parts that originates from glucotropaeolin; 2-phenylethyl isothiocyanate and benzenepropanenitrile derived from gluconasturtiin were found in root and bulb; 2-methoxyphenyl acetonitrile that comes from glucolimnanthin was found only in bulb. The side chains (R) of the identified aliphatic and aromatic glucosinolates derived in biosynthetic pathway involving the amino acids methionin and phenylalanine and corresponding chain elongation.

The identified sulfides dimethyl trisulfide (4.24-15.46%), methyl(methylsulfanyl)methyl disulfide (0.02-0.38%), and dimethyl tetrasulfide (0.16-1.41%), found in all plant parts, originate from *S*-methyl-L-cysteine sulfoxide (namely methiin). This non-volatile compound is characteristic for *Allium* species and thus the high percentages of degradation sulfur volatiles found in all volatile fractions were unusual. Obviously, next to the part of aminoacid cysteine, normally present in these plants involved in the biosynthesis of glucosinolates, part of cysteine is alkylated which is involved in biosynthestic pathway of *S*-methyl-L-cysteine formation. *S*-alkenyl-L-cysteine is oxidised and converted to *S*-methyl-L-cysteine sulphoxide.

Generally, enzyme aliinase is responsible for catalysing the hydrolysis of *S*-alk(en)yl-L-cysteine sulphoxide, whereby sulphenic acid, ammonia and pyruvate are formed. Condensation of sulphenic acid produces thermolabile thiosulphinates. Mixed polysulphides are formed from thiosulphinates during hydrodistillation process at 100 °C.

Table 2. Identified *S*-alk(en)yl cysteine sulphoxide degradation products in different plant parts of *Allium porrum*

Allium porrum L.			
***S*-alk(en)yl cysteine sulfoxide /** Identified degradation products	**Retention time (min)**	**Underground part (%)**	**Above ground part (%)**
Isoaliin, Methiin, Propiin			
2,4-Dimethyl thiophene	6.72	0.3	2.4
Methyl propyl disulfide	7.48	2.3	0.8
1,3-Dithiane	7.86	1.6	2.8
Dimethyl trisulfide	8.98	5.0	4.6
Dipropyl disulfide	15.19	2.9	1.1
trans-Propenyl propyl disulfide	15.64	2.6	0.9
Methyl(methylsulfanyl)methyl disulfide	16.65	1.1	1.2
Dimethyl tetrasulfide	20.67	0.6	1.2
(Methylsulfinyl)(methylsulfanyl)methane	24.76	2.1	0.4
Dipropyl trisulfide	25.34	6.1	1.7
trans-Propenylpropyl trisulfide	26.03	3.1	1.2

Dimethyl trisulphide was one of the most abundant volatile sulphur compound found in underground and aerial plant parts of *Allium porrum*, i.e. 5.0% and 4.6%, respectively. This volatile originates from the presence of *S*-methyl-L-cysteine sulphoxide (namely methiin). Dimethyl tetrasulphide, methyl(methylsulphanyl)methyl disulphide, and (methylsulphinyl)(methylsulphanyl)methane are also volatiles that confirm methiin presence as they represent the degradation compounds of this cystein sulphoxide. In the underground part of *A. porrum*, dipropyl trisulphide was found as the main volatile having 6.1%. This volatile originates from *S*-propyl-L-cysteine sulphoxide (namely propiin). Dipropyl disulphide is volatile that also confirms propiin presence.

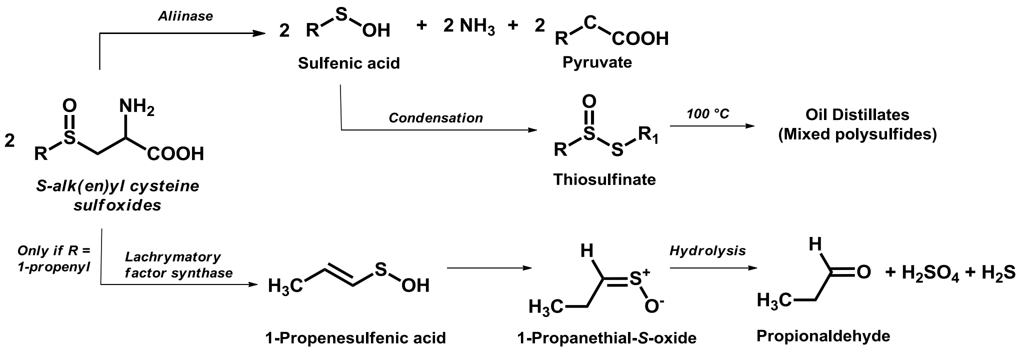


Figure 2. General scheme of *S*-alk(en)yl cystein sulfoxides degradation. R = $-CH_3$; $-CH_2CH_2CH_3$; $-CH=CH-CH_3$.

Other sulphur volatiles found in minor percentages represent volatiles of different cystein sulphoxide combination. Methyl propyl disulphide represents volatile formed from methiin and propiin. *trans*-Propenylpropyl disulphide and *trans*-propenylpropyl trisulphide, found in all parts represent volatile formed from the present propiin and unsaturated *S*-propenyl-L-cysteine sulfoxide (namely isoaliin). Di-1-propenyl disulphide can form various cyclic compounds including 2,4-dimethyl thiopene that was identified among volatiles obtained from aerial and underground part with 2.4%, and 0.3%, respectively.

Heavy metals analysis

The heavy metals analysis (Mo, Cd, Pb, Al, Mn, Fe, Co, Ni, Cu, Zn, As, and Se) of kohlrabi and leek is given in Table 3. The results showed that a huge amount of aluminium (Al) was incapsulated in the underground parts of leek (root and bulb), and smaller amount was translocated to the above ground parts, unlike kohlrabi, were the concentration of aluminium was almost 2.5 times higher in the above ground parts. The highest concentration was measured in leaves (3300.7 $\mu g\ g^{-1}$). Aluminium toxicity is a global and serious threat to plants grown in acidic soil resulting in reducing the yield and quality of crops grown on acidic soils.

Table 3. Measured heavy metals concentration in kohlrabi and leek

Part used	Heavy metal (μg g^{-1})											
	Mo	Cd	Pb	Al	Mn	Fe	Co	Ni	Cu	Zn	As	Se
KOHLRABI (*Brassica rupestris* Raf. ssp. *gongyloides* (L.) Janch.)												
UNDERGROUND PART												
Root	0.635	0.155	1.544	2882.5	91.287	1227.5	1.374	12.862	7.118	20.694	0.477	/
AERIAL PARTS												
Bulb	0.429	0.093	1.981	2710.3	22.592	390.5	0.389	6.607	7.018	30.125	0.842	1.143
Stem	0.628	0.066	0.966	670.5	13.380	132.5	0.184	7.317	9.535	13.515	0.348	/
Leaves	2.419	0.073	4.917	3300.7	33.989	211.5	0.301	0.748	7.469	32.437	1.430	/
Flowers	1.127	0.135	0.206	329.7	29.992	107.1	0.194	0.768	7.528	51.173	0.818	/
LEEK (*Allium porrum L.*)												
UNDERGROUND PARTS												
Root+bulb	0.804	0.169	0.919	1518.6	30.156	721.1	0.460	2.986	6.593	36.421	/	/
AERIAL PART												
Leaves	1.393	0.158	0.401	464.9	28.067	116.8	0.123	1.156	4.482	20.957	/	/

It causes many serious problems to plants by affecting their root, shoot, and leaf growth, accumulating callose, distorting the cytoskeleton, and disturbing the surface charge of plasma membranes, interferes with cell division in root tips and lateral roots, increases cell wall rigidity by cross linking pectins, fixes phosphorous in less available forms in soils and on root surfaces, decreases root respiration, interferes with enzyme activity governing sugar phosphorylation and the deposition of cell wall polysaccharides, and the uptake, transport, and also use of several essential nutrients (Ca, Mg, K, P and Fe) (Imadi *et al*., 2016). Aluminium toxicity cannot be prevented because it is one of the most common minerals found in Earth's crust. Although is toxic for many plants when the concentration is greater than 2 - 3 μg g^{-1} with a soil pH < 5.5, aluminium is also one of the most economically important toxic metals. The analysis of soil where the kohlrabi and leek were grown showed that the soil can be categorised as neutral or slightly alkaline soil (pH value determined was 7.63). Most plants contain no more than 0.2 mg g^{-1} dry mass Al. However, Al accumulator plants may contain over 10 times more Al without any injury. Tea plants (*Camellia sinensis* L.) are typical Al accumulators: the Al content in these plants can reach as high as 30 mg g^{-1} dry mass in old leaves (Mossor – Pietraszewska, 2001).
The aluminium concentration measured was high enough in both plants investigated and this could be one of the reasons why both plants looked non-developed i.e. dwarfed-liked. The aluminium concentration detected could point to conclusion that kohlrabi and leek are at least aluminium tolerant nonhyperaccumulator species and may be investigated as potential aluminium-hypertolerant hyperaccumulator plant, in which metals are actively taken up through the root, and largely loaded into xylem for root to shoot transport. In the shoot, the metals are safely sequestered in vacuoles.
Although the plants have a unique and very important role in delivering selenium (Se) from the environment to living systems, the selenium concentration was measured only in kohlrabi bulb with 1.143 μg g^{-1}. The rate of Se uptake depends on concentration and chemical form of Se in the soil solution, the rhizosphere conditions such as pH, and the presence of sulphate and phosphate, which compete with Se uptake. In plants, the Se metabolism is closely related to sulphur because of chemical similarities between the two elements (Handa *et al*., 2016).
The concentration of iron (Fe) measured in kohlrabi root was higher than permissible (1227.5 μg g^{-1}). Values measured in the above ground parts of kohlrabi were lower. The Fe wasn't translocated to other parts indicating the possibility that kohlrabi is a heavy metal-tolerant non-hyperaccumulator plant, in which iron can enter root cells where is sequestrated into root vacuoles, preventing translocation to shoots.
All the other concentrations of heavy metals were below permissible levels.

CONCLUSION

The investigated plants are sources of various volatile sulfur compounds (VSCs), which are breakdown products of glucosinolates and *S*-cystein-L-sulfoxides. *B. rupestris* was found to have alifatic and aromatic glucosinolates including sinigrin, glucoerucin, glucotropaeolin, gluconasturtiin, and glucolimnanthin, as well as *S*-methyl-L-cysteine sulphoxide (methiin). Characteristic volatile sulfur compounds of *A. porrum* were found to originate from *S*-cystein-L-sulphoxides that include methiin, propiin, and isoaliin. The wide range of VSCs found in the investigated plants and the diversity of their biological properties make them an excellent subject for further investigations in their biochemical and pharmacological activity.

The concentrations of heavy metals determined were in sequence Al > Fe > Mn > Zn > Cu > Ni > Pb > Mo > As > Co > Cd > Se for kohlrabi and Al > Fe > Mn > Zn > Cu > Ni > Mo > Pb > Co > Cd > As, Se for leek, respectively.

Aluminium and iron concentrations measured in leek, as well as measured iron concentration in kohlrabi indicate the possibility that these plants are a heavy metal-tolerant non-hyperaccumulator plants, in which aluminium and iron can enter root cells where are sequestrated into root vacuoles, preventing translocation to shoots. Measured aluminium concentrations in all parts of kohlrabi indicate that kohlrabi may be aluminium-hypertolerant hyperaccumulator plant, in which metals are actively taken up through the root, and largely loaded into xylem for root to shoot transport.

ACKNOWLEDGMENTS

This research has been fully supported by the Croatian Science Foundation under the projects BioSMe (HRZZ-IP-06-2016-1316; PI IB) and MEBTRACE (HRZZ-IP-09-2014-7530; PI DO).

Author contribution: MNM, MB, DO, SS, and IB conceived and planned the experiments. MNM, DO, TK performed experiments dealing with heavy metals analyses, MB, LR measured pH of soil, while AĐ, AS and IB performed experiments dealing with volatile sulfur compounds analyses. MNM, SS, AĐ, and IB drafted the manuscript with the input of all authors.

REFERENCES

Iranshahi, M. (2012): A review of volatile sulfur-containing compounds from terrestrial plants: biosynthesis, distribution and analytical methods. Journal of Essential Oil Research 4: 393–434.

Blažević, I., Montaut, S., Burčul, F., Rollin, P. (2016): Glucosinolates: Novel sources and biological potential. Glucosinolates, Springer International Publishing, New York, pp: 1–58.

Finley, J. W., Sigrid-Keck, A., Robbins, R. J., Hintze, K. J. (2005): Selenium Enrichment of Broccoli: Interactions between Selenium and Secondary Plant Compounds. The Journal of Nutrition 135 (5): 1236–1238.

Banerjee, S., Parasramka, M. A., Sarkar, F. H. (2012): Cellular, Molecular and Biological Insight into Chemopreventive and Therapeutic Potential of 3,3'-Diindolylmethane (DIM). Nutraceuticals and Cancer, Springer Netherlands, pp. 111–33.

Chase, W. M., Reveal, L. J., Fay, F. M. (2009): A subfamilial classification for the expanded asparagalean families Amaryllidaceae, Asparagaceae and Xanthorrhoeaceae. Botanical Journal of the Linnean Society 161: 132–136.

Rascio, N., Navari-Izzo, F. (2011): Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? Plant Science 180: 169–181.

Lin, Y.-F., Aarts, M. G. M. (2012): The molecular mechanism of zinc and cadmium stress response in plants. Cellular and Molecular Life Sciences 69: 3187–3206.

Anjum, N. A., Ahmad, I., Pereira, M. E., Duarte, A. C., Umar, S., Khan, N. A. (2012): The Plant Family Brassicaceae: Contribution Towards Phytoremediation. Springer Dordrecht Heidelberg New York London.

Ilijanić, M. and Gračanin, Lj. (1977): Uvod u ekologiju bilja. Školska knjiga, Zagreb.

Ansari, A. A., Gill, S. S., Gill, R., Lanza, G. R., Newman, L. (2015): Non-remediation Options. Phytoremediation. Management of Environmental Contaminants. Vol. 1: 164.

Blazevic, I., Males, T., Ruscic, M. (2014): Glucosinolates of *Lunaria annua*: thermal, enzymatic, and chemical degradation. Chemistry of Natural Compounds 49 (6): 1154–1157.

Imadi, S. R., Waseem, S., Kazi, A. G., Azooz, M. M., Ahmad, P. (2016): Aluminum Toxicity In Plants: An Overview. Plant Metal Interaction Emerging Remediation Techniques, Elsevier, pp: 2–14.

Handa, N., Bhardwaj, R., Kaur, H., Poonam, Kapoor, D., Rattan, A., Kaur, S., Kumar Thukral, A., Kaur, S., Arora, S., Kapoor, N. (2016): Selenium: An Antioxidative Protectant In Plants Under Stress. Plant Metal Interaction Emerging Remediation Techniques, Elsevier, pp: 180–181.

Mossor-Pietraszewska, T. (2001): Effect of aluminium on plant growth and metabolism, Acta Biochimica Polonica 48 (3): 673–686.

THE EFFECT OF STORAGE TIME DURING 6, 9 AND 12 MONTHS ON SHELF LIFE OF SPREADS BASED ON OILSEEDS AND NUTS*

UTICAJ VREMENA ČUVANJA TOKOM 6, 9 I 12 MESECI NA ODRŽIVOST MASNIH NAMAZA NA BAZI SEMENA ULJARICA I ORAHA

Vesna Vujasinović[1], Selma Čorbo[2], Sanja Dimić[3], Kristian Pastor[4], Miloš Bjelica[4], Etelka Dimić[4], Zoran Herceg[5]

Original scientific paper

Summary

Fat based spreads from nuts and oilseeds are popular food products with high oil contents, prepared without the addition of water. This study included the investigation of the most relevant parameters necessary to determine the fat spreads' stability during storage of 6, 9 and 12 months, with the aim to determine their optimal shelf life. Investigations were carried out with spreads (butter) commercially available in the market of the Republic of Serbia, made from sunflower kernels, naked pumpkin seed, sesame seed, roasted peanut, and walnut. The fatty acid composition of spread's oil phase was determined and the change of stability was monitored by measuring peroxide value, totox value and the acid value.

Key words: *fat spreads, shelf life, fatty acids, peroxide value, totox value, acid value*

Rezime

Složeni masni namazi na bazi orašastih plodova i semena uljarica su popularni prehrambeni proizvodi koje karakteriše visok sadržaj ulja, a pripremaju se bez dodatka vode. Ovaj rad obuhvata ispitivanja najvažnijih parametara, kako bi se sagledala stabilnost masnih namaza u period od 6, 9 i 12 meseci, u cilju definisanja njihovog optimalnog roka trajanja. Istraživanja su sprovedena na uzorcima komercijalnih masnih namaza (biljnih putera) sa tržišta Republike Srbije, pripremljenih od jezgra suncokreta, semena tikve golice, semena susama, pečenog arašida i jezgra oraha. Ispitan je sastav masnih kiselina masne faze namaza, a promena stabilnosti je praćena određivanjem peroksidnog broja, totoks vrednosti i kiselinskog broja.

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina

[1] The College of Hotel Management, Kneza Višeslava 70, 11030 Belgrade, Serbia
[2] Faculty of Agriculture and Food Sciences, University of Sarajevo, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina
[3] Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia
[4] University of Novi Sad, Faculty of Technology, Bul. cara Lazara 1, 21000 Novi Sad, Serbia
[5] University of Zagreb, Faculty of Food Technology and Biotechnology, Pierrotijeva 6, 10000 Zagreb, Croatian

Ključne reči: *masni namazi, održivost, masne kiseline, peroksidni broj, totox vrednost, kiselinski broj*

INTRODUCTION

Nut butters and spreads are spreadable products made from various nuts that are ground into paste. Both nut products can be spread like commercially available butter. They can be produced from almond, hazelnut, cashew, peanut, pistachio and walnut. Similar spreads can also be made from some oilseeds, such as sesame seed, pumpkin seed, soybean and sunflower seed. Roasting and milling (particle size reduction) are two important stages for the production of these spreads that affect the textural and rheological characteristics and overall quality of the spreads (Shakerardekani *et al.,* 2013; Radočaj *et al.,* 2012; Dimić *et al.,* 2013; Dimić *et al.,* 2016).
Oil seeds and tree nuts, as well as their oils, contain a number of bioactive and health-promoting components (Miraliakbari and Shahidi, 2008; Alasavar and Shahidi, 2009; Rabrenović *et al*., 2014). In addition, they are highly nutritious and provide macronutrients, such as fat, protein, and carbohydrates, but also lipophilic bioactive components (essential fatty acids, phospholipids, phytosterols, tocopherols, squalen, *etc.*), and phytochemicals (phenolic acids, flavonoids, lignans, carotenoids, terpenes, phytoestrogens, *etc*.) (Venkatachalam and Sathe, 2006). Moreover, tree nuts and oilseeds contain numerous types of antioxidants with different properties (Blomhoff *et al.,* 2006). Nuts (tree nuts and peanuts) are now positioned with meat, poultry, fish, dry beans, and eggs in the U.S. Food Guide Pyramid. Therefore, they constitute one of the most nutritionally concentrated type of food available.
Similar to other high oil content products, nut spreads are susceptible to autoxidation. The oxidation process of these products can be controlled by the application of antioxidants, as well as by using processing techniques that minimize tocopherol and other natural antioxidants losses (Shakerardekani *et al.,* 2013).
The objective of this study was to characterize the fatty acid composition and the change of stability of fat spreads, based on different oilseeds and walnut, commercially available in the market of the Republic of Serbia. The shelf life of spreads was monitored by measuring peroxide value, Totox value, and the acid value.

MATERIALS AND METHODS

Materials

Five different fat spreads of plant origin, labelled as: sunflower butter, pumpkin butter, sesame butter, peanut butter and walnut butter, were evaluated in this study. All spreads were purchased randomly from a local supermarket. The spreads were packaged in small glass jars, with a net weight of 190 g (sesame seed and walnut butter) and 200 g (sunflower seed, peanut and hull-less pumpkin seed butter), and had a declared shelf life of 12 months. The collected fat spreads were stored in the original package at room temperature without a direct sunlight exposure, and the analysis were carried out periodically, i.e. after 0, 6, 9 and 12 months.

Methods

Fatty acid composition
Fatty acid methyl esters (FAME) were prepared according to the standard method (SRPS EN ISO 12966-2:2011) and the fatty acid profile was determined using a gas chromatography method (SRPS EN ISO 15304:2009) on a Hewlett-Packard series II^{plus} GC analyzer, equipped with an automatic sampler HP 7673, splitless injector, flame-ionization detector and integrator. The oil phase was extracted from spreads using a cold extraction method (Dimić and Turkulov, 2000).

Change of stability during storage
For the investigation of chemical parameters, which describe changes of quality during storage, the following standard methods were applied: Acid value - AV (mgKOH/g) SRPS EN ISO 660:2011, Peroxide value - PV (mmol/kg) SRPS EN ISO 3960:2011, and *para*-Anisidine value - AnV SRPS EN ISO 6885:2011. Totox value was calculated as: Totox = 2PV + AnV (Dimić and Turkulov, 2000).
The experimental values were expressed as means of three determinations with standard deviation.

RESULTS AND DISCUSSION

Fatty acid composition of spreads
The fatty acid composition of the investigated spreads (Table 1) was very different but typical for the oilseeds and walnut, that were used as the main ingredients. Essential linoleic C18:2 omega-6 fatty acid was found in all spreads, regardless of the spread type, in the range between 9.70±0.02% (peanut butter) and 59.6±0.5% (walnut spread). However, polyunsaturated alpha-linolenic C18:3 omega-3 fatty acid was detected only in the walnut spread in a relatively high amount of 12.9±0.03%.
The absence of *trans* fatty acids is one of the major nutritional qualities of these spreads. Lower SAFA content is also desirable in vegetable oils and food, due to their undesirable health effects. The highest content of total SAFA was found in the sesame (19.9%) and pumpkin seed (19.5%) spreads, while the content of palmitic fatty acid, which is found to cause atherosclerosis, was around 13% in both spread samples.
Both sunflower and peanut butter spreads had total SAFA content much lower than in sesame and pumpkin seed spreads (30% lower), while walnut spread had about 50% lower palmitic fatty acid content compared to sunflower and peanut butter spreads. Palmitic fatty acid content in sunflower, walnut and peanut butter spreads was around 7%.

Tab. 1. Principal fatty acids, *trans* fatty acids and fatty acids' ratios in the oil phase of investigated spread samples

Fatty acid (% of Σ FAME)	Sunflower	Pumpkin	Sesame	Peanut	Walnut
C16:0	7.1±0.06	13.0±0.04	13.4±0.07	7.1±0.02	7.5±0.02
C18:0	6.4±0.03	6.5±0.09	6.8±0.06	2.2±0.01	2.8±0.01
C18:1	31.7±0.05	38.1±0.09	32.8±0.09	72.8±0.3	17.2±0.03
C18:2	54.6±0.10	42.4±0.04	45.4±0.10	9.7±0.02	59.6±0.5
C18:3	nd*	nd	0.5±0.0	0.1±0.0	12.9±0.03
Σ *trans*	nd	nd	nd	nd	nd
Σ SAFA**	13.7	19.5	19.9	13.9	10.3
Σ MUFA***	31.7	38.1	32.8	76.4	17.2
Σ PUFA****	54.6	42.4	45.4	9.7	72.5

* not detected, ≤0.05%
**SAFA - saturated fatty acids
***MUFA - monounsaturated fatty acids
****PUFA - polyunsaturated fatty acids

It is also important to emphasize that the omega-6/omega-3 fatty acid ratio is 4.62 in the walnut spread, which is in the range recommended by the newest nutritional guidelines (Dubois *et al.*, 2007; Brinkman *et al.*, 2011; Schaefer, 2002). A very similar ratio of omega-6/omega-3 fatty acids in walnut oil was found by Radočaj and Dimić (2013), while in the sesame and pumpkin seed oils these values were very high (199.8 and 70, respectively), which indicated that omega-3 alpha-linolenic acid was present in these oils in negligible amounts.

Change of stability of spreads during storage

The change in acidity of investigated spreads during storage in the period of 12 months is presented in Figure 1. The most significant and permanent change in the acid value (AV) was observed in the sample of peanut butter, whereby AV with an initial value of 2.06±0.42 mgKOH/g increased to 4.22±0.06 mgKOH/g, after 12 months of storage. The increase of the acidity during storage was 104% in peanut butter and 60% in sunflower seed butter, while in other analyzed spreads the changes were no significant.

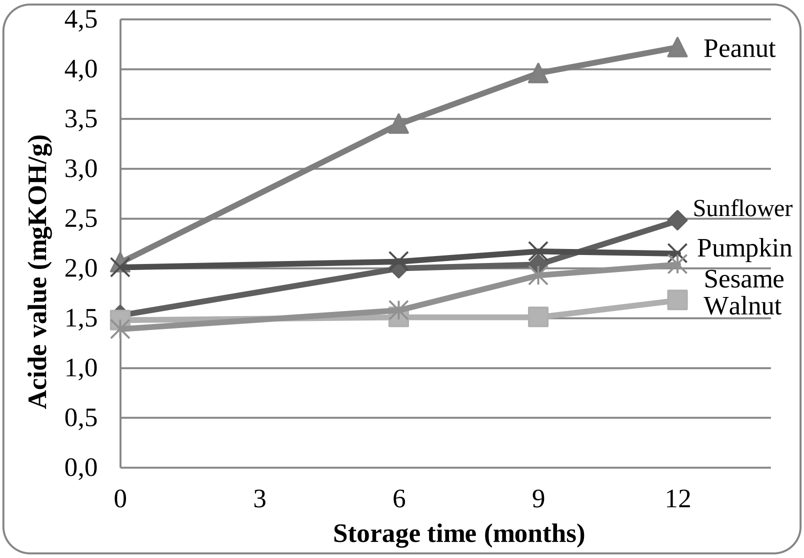


Fig. 1. The changes of acid values of the investigated spreads during storage time of 12 months

The changes in the content of primary (PV), as well as the total oxidation products (Totox) in the analyzed spreads during the investigated storage time is shown in Figures 2 and 3, respectively.

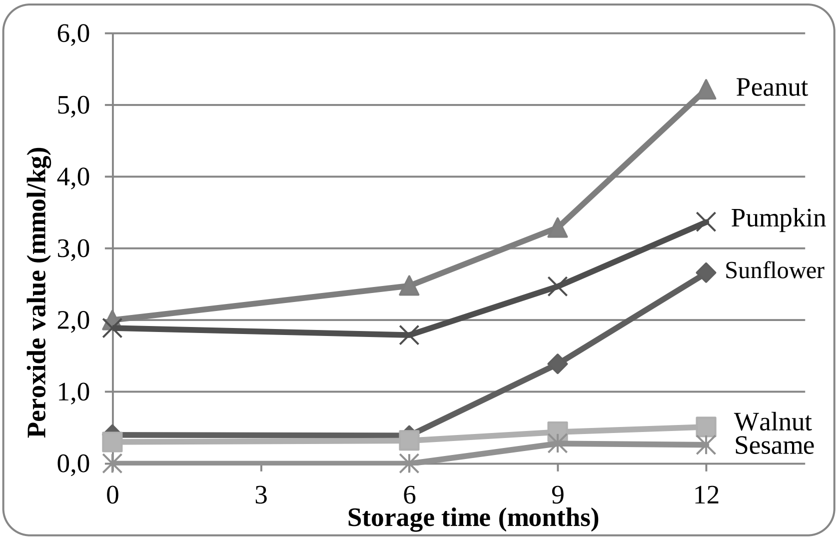


Fig. 2. The changes of peroxide values of the investigated spreads during storage time of 12 months

The increase of PV during the storage time indicated the decrease of oxidative stability of spreads. However, it can be said that the PV changes were insignificant in all investigated spreads in the storage period of the first 6 months. After this time, the oxidation of the oil phase started with different intensities, depending on the type of spread. The highest PV after 12 months was found in peanut butter, 5.22±0.02 mmol/kg, and the lowest in the spread of sesame seeds, 0.26±0.01 mmol/kg. The relatively small increase in PV in the sample of walnut spread was surprising, since the share of PUFA in this spread exceeds 70%. However, walnut is naturally rich in phenolic type antioxidants, that contribute largely to the protection from oxidation (Shahidi and Miraliakbari, 2005).

The content of total oxidation products, expressed as PV and *p*-AnV, is also increased after 6 months in all investigated spreads, but very differently. The highest content was determined in the samples of peanut and pumpkin seed spreads, 10.99±0.02 and 9.89±0.05, respectively.

Of all analyzed spread samples, the worst stability has been detected in the sample of peanut butter, although, according to the literature data (Woodroof, 1966), this spread should be characterized by a good stability. It is most probable that the quality of the raw material used for the production of this spread was not very good. The Totox value of the fresh sample of this spread was 4.33±0.26.

The best quality regarding the oxidative stability was maintained in sesame and walnut spreads, where the Totox values after 12 months were 0.85±0.02 and 2.04±0.03, respectively.

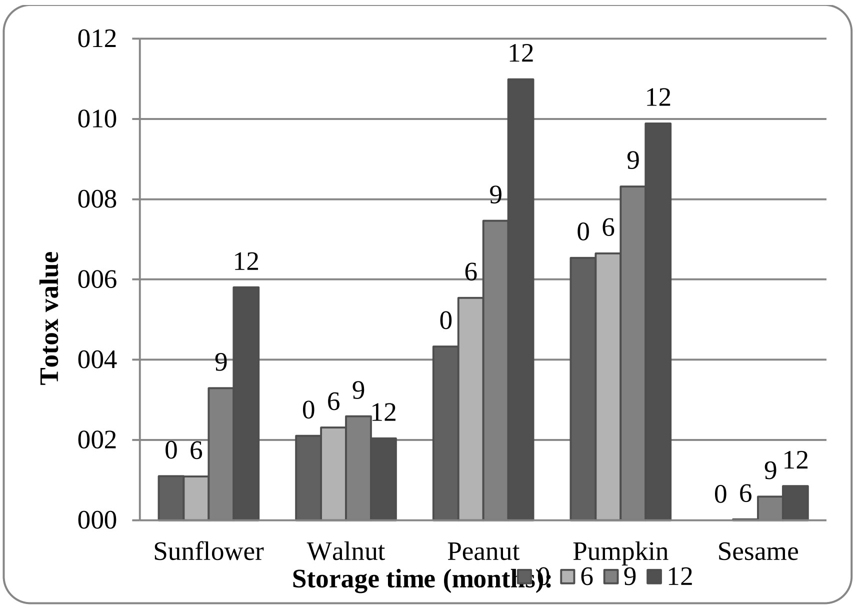


Fig. 3. The changes of Totox values of the investigated spreads during storage time of 12 months

CONCLUSIONS

The fatty acid composition of the analyzed spreads is very different but typical for the oilseeds and walnut, that were used as the main ingredients. The major part of unsaturated fatty acids, which contribute to the biological value of the spread, did not cause a significantly higher degree of oxidation at room temperature. It is possible to claim that the optimum stability of the investigated spreads, in terms of the minimal change in acidity and oxidation products, is up to 6 months. However, the declared shelf life of one year is also satisfying.

REFERENCES

Alasalvar, C., Shahidi, F. (2009). Natural antioxidants in tree nuts. Eur. J. Lipid Sci. Technol., Vol. 111, No. 11, pp. 1056-1062.

Blomhoff, R, Carlsen, M. H., Andersen, L. F., Jacobs Jr., D. R. (2006). Health benefits of nuts: potential role of antioxidants. Br J Nutr., Vol. 96 (Suppl. 2), pp. 52S-60S.

Brinkman, M. T., Karagas, M. R., Zens, M. S., Schned, A. R., Reulen, R. C., Zeegers, M. P. (2011). Intake of α-linolenic acid and other fatty acids in relation to the risk of bladder cancer; results from the new Hampshire case-control. British Journal of Nutrition, Vol. 106, No. 7, pp. 1070-1077.

Dimić, E., Turkulov, J. (2000). Kontrola kvaliteta u tehnologiji jestivih ulja. Univerzitet u Novom Sadu, Tehnološki fakultet, Novi Sad.

Dimić, E., Vujasinović, V., Kravić, S., Borić, B. (2016). Energy value and fatty acid profile of commercial fat spreads based on oilseeds in Serbian market. Uljarstvo, Vol. 47, No. 1, pp. 25-30.

Dimić, E., Vujasinović, V., Radočaj, O., Borić, B. (2013). Sensory evaluation of commercial fat spreads based on oilseeds and walnut. APTEFF, Vol. 44, No 1, pp. 21-30.

Dubois, V., Breton, S., Linder, M., Fanni, J., Parmentier, M. (2007). Fatty acid profiles of 80 vegetable oils with regard to their nutritional potential. Eur. J. Lipid Sci. Technol., Vol. 109, No. 7, pp. 710-732.

Miraliakbari, F., Shahidi, F. (2008). Lipid class compositions, tocopherols and sterols of tree nut oils extracted with different solvents. J Food Lipids, Vol. 15, No. 1, pp. 81-96.

Rabrenović, B., Dimić, E., Novaković, M., Tešević, V., Basić, Z. (2014). The most important bioactive components of cold pressed oil from different pumpkin (*Cucurbita pepo* L.) seeds, LWT- Food Science and technology, Vol. 55, No. 2, pp. 521-527.

Radočaj, O. and Dimić, E. (2013). Physico-chemical and nutritive characteristics of selected cold-pressed oils found in the European market. Rivista. Ital. Sost. Grasse, Vol. 90, No. 4, pp. 219-228.

Radočaj, O., Dimić, E., Vujasinović, V. (2012). Development of a hull-less pumpkin (*Cucurbita pepo* L.) seed oil press-cake spread. Journal of Food Science, Vol. 77, No. 9, pp. C1011-1017.

Schaefer, E. J. (2002). Lipoproteins, nutrition, and heart disease. American Journal of Clinical Nutrition, Vol.75, No. 2, pp. 191-212.

Shahidi, F., Miraliakbari, H. (2005). Tree nut oils. In Bailey's Industrial Oil and Fat Products, 6th ed.: Editor; Shahidi, F., Wiley-Interscience, Hoboken, NY, pp. 175-193.

Shakerardekani, A., Karim, R., Ghazali, H. M., Chin, N. L. (2013). Textural, rheological and sensory properties and oxidative stability of nut spreads – A review. International Journal of Molecular Sciences,Vol. 14, No. 2, pp.4223-4241.

Srpski standard SRPS EN ISO 12966-2:2011, Ulja i masti biljnog i životinjskog porekla - Gasna hromatografija metilestara masnih kiselina - Deo 2: Priprema metilestara masnih kiselina, Institut za standardizaciju, Beograd.

Srpski standard SRPS EN ISO 15304:2009, Ulja i masti biljnog i životinjskog porekla - Određivanje sadržaja *trans*-izomera masnih kiselina u biljnim uljima i mastima - Metoda gasne hromatografije, Institut za standardizaciju, Beograd.

Srpski standard SRPS EN ISO 660:2011, Ulja i masti biljnog i životinjskog porekla - Određivanje kiselinskog broja i kiselosti, Institut za standardizaciju, Beograd.

Srpski standard SRPS ISO 3960:2011, Ulja i masti biljnog i životinjskog porekla - Određivanje peroksidnog broja - Jodometrijsko (vizuelno) određivanje završne tačke, Institut za standardizaciju, Beograd.

Srpski standard SRPS EN ISO 6885:2011, Ulja i masti biljnog i životinjskog porekla — Određivanje anisidinskog broja, Institut za standardizaciju, Beograd.

Venkatachalam, M., Sathe, S. K. (2006). Chemical composition of selected edible nut seeds. J Agric Food Chem. Vol. 54, No. 13, pp. 4705-4714.

Woodroof, J. G. (1966). Peanuts – Production, Processing, Products. Avi Publishing Company, Inc., Westport, Connecticut, pp. 127-164.

FATTY ACID COMPOSITION AND BIOACTIVE COMPOUNDS OF COLD-PRESSED GRAPE SEED OILS FROM RED AND WHITE GRAPE CULTIVARS GROWN IN VOJVODINA*

SASTAV MASNIH KISELINA I BIOAKTIVNE KOMPONENTE HLADNO PRESOVANIH ULJA KOŠTICA CRNOG I BELOG GROŽĐA GAJENIH U VOJVODINI

Miloš Bjelica[1], Vesna Vujasinović[2], Selma Čorbo[3], Sanja Dimić[4], Kristian Pastor[1]

Original scientific paper

Summary

Grape seed oil is gaining a growing popularity as a culinar oil, and has been studied as a possible source of specialty lipids. In this study, the samples of cold-pressed oil obtained from naturally dried seeds of two different red (Merlot and Hamburg) and two white (Italian Riesling and Sila) grape varieties, grown in Vojvodina (Serbia), were characterized by determining fatty acid composition and nutritive profile parameters. Linoleic acid was the most abundant essential fatty acid in all analysed oils, contributing between 73.60±0.69% and 85.59±0.05% of total fatty acids. The content of total saturated fatty acids was below 10%, while mono- and polyunsaturated fatty acids totalled up to 16.37% and 85.79%, respectively. In addition to essential fatty acids, investigated cold-pressed oil samples were rich in other health-beneficial compounds, like tocopherols, from 27.81±2.20 mg/100g up to 57.52±4.46 mg/100g, and phenolics, from 8.09±0.56 to 12.33±0.21 mgGAE/kg. Overall, the results suggest no differences between red and white grape seed oils, but between the investigated grape varieties. In terms of its nutritional profile cold-pressed grape seed oil can be highly recommended in nutrition.

Key words: *cold-pressed grape seed oil, fatty acid composition, tocopherols, phenols*

Rezime

Ulje koštica grožđa je sve popularnije kao salatno ulje i široko se ispituje kao poseban izvor specijalne vrste ulja. U ovom radu data je karakterizacija, na bazi sastava masnih kiselina i parametara nutritivnog profila, uzoraka hladno presovanih ulja proizvedenih

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina

[1] University of Novi Sad, Faculty of Technology, Bul. cara Lazara 1, 21000 Novi Sad, Serbia
[2] The College of Hotel Management, Kneza Višeslava 70, 11030 Belgrade, Serbia
[3] Faculty of Agriculture and Food Sciences, University of Sarajevo, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina
[4] Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia

od prirodno osušenih koštica crnog (sorte Merlot i Hamburg) i belog (sorte Italijanski Rizling i Sila) grožđa kultivisanih u Vojvodini (Srbija). Najzastupljenija masna kiselina u svim uzorcima analiziranih ulja bila je linolna, u količini od 73,60±0,69% do 85,59±0,05%. Sadržaj zasićenih masnih kiselina je bio ispod 10%, dok je sadržaj mono- i polinezasićenih masnih kiselina iznosio do 16,37% i 85,79%, respektivno. Osim esencijalnih masnih kiselina, ispitana ulja su bila bogata i drugim nutritivno visokovrednim sastojcima kao što su tokoferoli, od 27,81±2,20 mg/100g do 57,52±4,46 mg/100g, i fenoli od 8,09±0,56 do 12,33±0,21 mgGAE/kg. Dobijeni rezultati su ukazali na to da razlika između ulja koštica crnog ili belog grožđa ne postoji, već razlika postoji u odnosu na sortu grožđa. S obzirom na nutritivni profil hladno presovano ulje koštica grožđa se može posebno preporučiti u ishrani.

Ključne reči: *hladno presovano ulje koštica grožđa, sastav masnih kiselina, tokoferoli, fenoli*

INTRODUCTION

Grapes are mainly utilized for their juice, which is used primarily in wine-making. Grape seed oil is produced from the seeds in the pomace left over from juice and wine production, and thus adds value to the industry. This also helps to reduce waste disposal problems. Grape seed oil is gaining a growing popularity as a culinary oil, and has been studied as a possible source of specialty lipids. It is a rich source of linoleic acid (Beveridge *et al.*, 2005; Lutterodt *et al.*, 2011; Bjelica and Vujasinović, 2017), which is associated with promotion of cardiovascular health by down-regulating low-density lipoprotein cholesterol (LDL-C) production, and enhancing its clearance (Wijendran and Hayes, 2004). Cold-pressing is a method of oil extraction that involves no heat or chemical treatment, and hence may retain more health beneficial components in oil, such as natural antioxidants (Yu *et al.*, 2005; Radočaj and Dimić, 2013). The cold-pressed oils may also be a better source of beneficial components, such as phenolic compounds (Bail *et al.*, 2008; Vujasinović *et al.*, 2017), as well as some other health-beneficial phytochemicals (Crews *et al.*, 2006). Although the yield of oil is usually lower than that with conventional organic solvent extraction, there is no concern about solvent residues in the oil, making it as a safer and more consumer-desired product (Matthäus and Spener, 2008).
The objective of this study was to characterize nutritive quality of cold-pressed grape seed oils from different grape varieties grown in Autonomous Province of Vojvodina (Serbia).

MATERIALS AND METHODS

Materials

Cold-pressed Merlot, Hamburg (red grape cultivars), Italian Riesling and Sila (white grape cultivars) grape seed oils were obtained from a local small scale oil mill. Air dried clean grape seeds from fresh juice (with moisture content of about 8%) were pressed on a small screw press (Koprulu Machine, Tip KYP20D, Turkey). The oil samples were decanted for 72 h to remove the residual sludge, formed naturally in crude oils. After this period, the oils were kept in a refrigerator at 6±1 ^{o}C until analysis.

Methods

Fatty acid composition. Fatty acid methyl esters (FAME) were prepared according to the standard method (SRPS EN ISO 12966-2:2011) and the fatty acid profile was analysed by a gas chromatography method (SRPS EN ISO 15304:2009) on a Hewlett-Packard series II^{plus} GC analyzer, equipped with an automatic sampler HP 7673, splitless injector, flame-ionization detector and integrator.

The content of total tocopherols and tocotrienols was determined by the spectrophotometric method according to Emmerie-Engel (Pacqout *et al.*, 1967).

Total phenolic contents-TPC of the seed oils were measured using Folin-Ciocalteu reagent, according to the method described by Haiyan *et al.* (2007).

Relative DPPH-scavenging capacity was assessed against the stable 2,2-diphenyl-1-picrylhydrazyl radical (Martinez and Maestri, 2008).

The content of total carotenoids was determined by measuring the absorbance of oil solution in cyclohexane at 445 nm using the British Standard method (1997). This method considers β-carotene as a carotenoid equivalent.

The content of total chlorophylls was estimated according to the method described by Pokorny *et al.* (1995) and it was presented as pheophytin-a, by measuring absorbance of the oil dissolved in chlorophorm at 667 nm using UV/VIS spectrophotometer model T80+ (PG Instruments Limited, London).

Phospholipid content of oils was determined by AOCS Official method (Ca 12-55, 2009).

RESULTS AND DISCUSSION

Fatty acid composition of cold-pressed grape seed oils

Linoleic acid was the most abundant fatty acid in all four samples of analyzed cold-pressed grape seed oils, contributing between 73.60±0.69% and 85.59±0.05% of total fatty acids (Table 1). Sila seed oil (white grape cultivar) had the highest content of linoleic acid (85.59%), while the Hamburg contained the lowest amount (73.6%). The grape seed oils also contained significant amounts of oleic acid, ranging from 9.40±0.01% (Sila) to 16.25±0.30% (Hamburg). Other fatty acids present in oils include stearic acid (about 1.5 to 3.5%) and palmitic acid (about 3-6%). The content of total saturated fatty acids was below 10%, while mono- and polyunsaturated fatty acids totalled up to 16.37% and 85.79%, respectively (Table 1). These results are in accordance with similar ranges reported in the literature (Beveridge *et al.*, 2005; Crews *et al.*, 2006; Lutterodt *et al.*, 2011). Beveridge *et al.* (2005) reported a linoleic acid range of 66.8-73.6% in the seed oils of seven different varieties of grapes.

They also reported oleic acid values ranging from 12% to 19%, with palmitic and stearic acids ranging within 6.28-8.62% and 3.6-5.26%, respectively. Some authors have, however, reported higher or lower values, depending on seed origin and method of oil extraction. El-Shami *et al.* (1992) reported higher oleic acid contents in Egyptian grape seeds. The results from Crews *et al.* (2006) showed different proportions of fatty acids present in grape seed oils from France, Italy and Spain. Ovcharova *et al.* (2016) reported about the changes that occur in the fatty acid composition of developing seeds of Bulgarian grape variety. They found that the content of the essential linoleic acid in the triacylglycerol fraction during the whole period of vegetation increased from 49.8% to 72.3%, oleic from 15.9% to 17.3%, but palmitic acid decreased from 24.1% to 8.8%.

Tab. 1. Fatty acid profile of cold-pressed grape seed oils (g/100g)*

Fatty acid	Merlot	Hamburg	Italian Riesling	Sila
C 16:0	3.56±0.11	6.34±0.21	3.42±0.02	3.12±0.00
C 16:1	nd	nd	0.06±0.01	nd
C 18:0	1.80±0.02	3.39±0.13	2.15±0.05	1.50±0.01
C 18:1	9.72±0.25	16.25±0.30	13.81±0.05	9.40±0.01
C 18:2	84.51±0.31	73.60±0.69	80.21±0.18	85.59±0.05
C 18:3	0.20±0.03	0.22±0.01	0.19±0.03	0.20±0.01
C 20:0	0.12±0.03	0.09±0.01	0.07±0.01	0.08±0.01
C 20:1	0.09±0.02	0.12±0.01	0.08±0.01	0.11±0.01
SFA	5.48±0.10	9.81±0.35	5.64±0.08	4.70±0.02
MUFA	9.81±0.23	16.37±0.32	13.96±0.07	9.51±0.02
PUFA	84.71±0.34	73.82±0.68	80.40±0.15	85.79±0.04

*Data are expressed as means ± standard deviation (n=3), SFA, MUFA and PUFA stand for saturated, monounsaturated and polyunsaturated fatty acids, respectively.

Bioactive profile of cold-pressed grape seed oils

The contents of bioactive compounds of investigated cold-pressed grape seed oils are presented in Table 2. The highest and the lowest contents of total tocopherols and tocotrienols were found in cold-pressed oil samples produced from the seeds of red grape varieties, Merlot 57.52±4.46 mg/100 g, and Hamburg 27.81±2.20 mg/100g. Tocol contents in the analysed oil samples of white grape varieties was approximately 45 mg/100g. According to Beveridge *et al.* (2005) the total content of tocopherols and tocotrienols in eight samples of cold-pressed grape seed oil was between 60 and 100 mg/100g, while the total content of these compounds in the grape seed oil samples from three different countries was from 6 to 121 mg/100g (Crews *et al.*, 2006). This indicates that a significant variations in total tocopherol and tocotrienol contents exist between various grape varieties, as well as different geographic origins of cultivation.

Total phenolic contents in the grape seed oils in this study ranged from 8.09±0.56 mgGAE/kg (Italian Riesling) to 12.33±0.21 mgGAE/kg (Hamburg). This TPC values are similar to the results published by Pardo *et al.* (2009) (10.68 to 34.43 mg CAE/kg), but much lower than those reported by Lutterodt *et al.* (2011) (0.16-0.80 mgGAE/g). The low solubility of low molecular weight phenolics in the oil, combined with the binding of some phenolic acids to the seed material, may explain the low TPC values in these oils.

When it comes to pigments, the total contents of chlorophylls (from 29.57±0.01 to 67.44±0.00 mg/kg) in all analyzed cold-pressed grape seed oil samples was much higher compared to β-carotene contents (from 0.22±0.01 to 16.75±0.04 mg/kg). The phospholipid content is significantly lower in this type of cold-pressed oil. The highest content of 133.5±18.4 mg/kg was found in the oil sample of a white grape variety - Sila.

Obvious differences in antiradical activities are observed between the individual grape varieties, but this factor is not a significant indicator of a red or white grape variety.

Tab. 2. Contents* of bioactive compounds of cold-pressed grape seed oils

Compound	Merlot	Hamburg	Italian Riesling	Sila
Tocopherols** (mg/100g)	57.52±4.46	27.81±2.20	46.40±2.57	43.88±5.37
Phenolics (mgGAE/kg)	8.73 ± 0.80	12.33±0.21	8.09 ± 0.56	9.02 ± 0.21
β-carotene (mg/kg)	0.22±0.01	16.75±0.04	0.25±0.01	0.36±0.00
Chlorophylls (mg/kg)	33.70±0.00	46.78±0.01	29.57±0.01	67.44±0.00
Phospholipids (mg/kg)	68.7±5.95	36.55±3.95	28.68±0.95	133.5±18.4
DPPH activity EC_{50} (mg/mg)	321.30±0.59	144.86±1.00	241.46±1.48	188.67±1.40

*Data are expressed as means ± standard deviation (n=3); **Total content of tocopherols and tocotrienols

CONCLUSIONS

Utilization of agro-industrial waste material is an excellent way of adding value to crop production and processing industries, with the added advantage of reducing waste disposal problems. Wine and juice manufacturing produces tons of grape seeds as by-products. The investigated cold-pressed grape seed oils from red and white grape cultivars grown in Vojvodina are rich in the essential omega 6 fatty acid and other health-beneficial compounds, like tocopherols and phenolics. In terms of a nutritional profile cold-pressed grape seed oil can be highly recommended in nutrition.

REFERENCES

AOCS Official methods and recommended practice of the American Oil Chemists' Society (2009). Method Ca 12-55, AOCS Press, Champaign, IL.

Bail, S., Stuebiger, G., Krist, S., Unterweger, H., Buchbauer, G. (2008). Characteristics of various grape seed oils by volatile compounds, triacylglycerol composition, total phenols and antioxidant capacity. Food Chemistry, Vol. 108, No. 4; pp. 1122-1132.

Beveridge, T. H. J., Girard, B., Knopp, T., Drover, J. C. G. (2005). Yield and composition of grape seed oils extracted by supercritical carbon dioxide and petroleum ether: Varietal effects. Journal of Agricultural and Food Chemistry, Vol. 53, No. 5; pp. 1799-1804.

Bjelica, M., Vujasinović, V. (2017). Uporedna karakterizacija sastava i nutritivne vrednosti hladno presovanog i rafinisanog ulja semenki grožđa. Zbornik radova XXII Savetovanja o biotehnologiji sa međunarodnim učešćem, 10-11. mart, Čačak, pp. 521-526.

British Standard methods of analysis of fats and fatty oils (1997). Other methods. Determination of carotene in vegetable oils. British Standards Illustrations, London (BS 684-2.20).

Crwes, C., Hough, P., Godward, J., Brereton, P., Lees, M., Guiet, S. (2006). Quantification of the main constituents of some authentic grape seed oils of different origin. Journal of Agricultural and Food Chemistry, Vol. 54, No. 17; pp. 6261-6265.

El-Shami, S. M., El-Mallah, M. H., Mohamed, S. S. (1992). Studies on the lipid constituents of grape seeds recovered from pomace resulting from white grape processing. Grassas y Aceites, Vol. 43, No. 3; pp. 157-160.

Haiyan, Z., Bedgood, Jr. D. R., Bishop, A. G., Prenzler, P. D., Robards, K. (2007). Endogenous biophenol, fatty acid and volatile profiles of selected oils. Food Chem., Vol. 100, No. 4; 1544-1551.

Lutterodt, H., Slavin, M., Whent, M., Turner, E., Yu, L. (2011). Fatty acid composition, oxidative stability, antioxidant and antiproliferative properties of selected cold-pressed grape seed oils and flours. Food Chemistry, Vol. 128, No. 2; pp. 391-399.

Martinez, M., Maestri D. (2008). Oil chemical variation in walnut (*Juglans regia* L.) genotypes grown in Argentina. Eur. J. Lipid Sci. Technol. 110, No. 12; pp. 1183-1189.

Matthäus, B., Spener, F. (2008). What we know and what we should know about virgin oils – a general introduction. Eur. J. Lipid Sci. Technol., Vol. 110, No. 7; pp. 597-601.

Ovcharova, T., Zlatanov, A., Ivanov, A. (2016). Changes in composition of grape seed oil during vegetation. La Rivista Italiana delle Sostanze Grasse, Vol. 93, No. 4; pp. 195-198.

Pokorny, J., Kalinova, L., Dysseler, P. (1995). Determination of chlorophyll pigments in crude vegetable oils. Pure and Appl. Chem. Vol. 67, No. 10; pp. 1781-1787.

Pardo, J. E., Fernandez, E., Rubio, M., Alvarruiz, A., Alonso, G. L. (2009). Characterization of grape seed oil from different grape varieties (*Vitis vinifera)*. Eur. J. Lipid Sci. Technol., Vol. 111, No. 1, pp. 188-193.

Paquot, C., Mercier, J., Lefort, D., Mathieu, A., Perron, R. (1967). Metode analize lipida. Poslovno udruženje proizvođača biljnih ulja, Beograd, pp. 175-179.

Radočaj, O., Dimić, E. (2013). Physico-chemical and nutritive characteristics of selected cold-pressed oils found in the European market. La Rivista Italiana delle Sostanze Grasse, Vol. 90, No. 3; pp. 219-228.

Vujasinović, V., Milovanović, N., Bjelica, M., Dimić, S., Pastor, K. (2017). Uticaj tehnološkog procesa proizvodnje na karakteristike susamovog ulja. Zbornik radova, 58. Savetovanje: Proizvodnja i prerada uljarica, 18-23, jun, Herceg Novi, Crna Gora, pp. 141-148.

Wijendran, V., Hayes, K. C. (2004). Dietary n-6 and n-3 fatty acid balance and cardiovascular health. Annual Review of Nutrition, Vol. 24, pp. 597-615.

Yu, L., Zhou, K., Parry, J. (2005). Antioxidant propreties of cold-pressed black caraway, carrot, cranberry, and hemp seed oils. Food Chemistry, Vol. 91, No. 4; pp. 723-729.

UTJECAJ NAČINA PRERADE NA SADRŽAJ POLIFENOLNIH KOMPONENTI U SOKOVIMA OD JABUKE *

EFFECT OF PROCESSING ON THE POLYPHENOLIC COMPOUNDS IN APPLE JUICES

Asima Akagić[1], Amila Vranac[1], Nermina Spaho[1], Fuad Gaši[1], Mirsad Kurtović[1], Mekjell Meland[2]

Original scientific paper

Rezime

Cilj rada je istražiti sadržaj polifenola u monokomponentnim i kupažiranim bistrim i mutnim sokovima od jabuke, proizvedenim sa i bez dodatka L-askorbinske kiseline. Radi poboljšanja polifenolnog profila sokova proizvedenih od komercijalnih kultivara jabuke, koje se dominantno uzgajaju u Bosni i Hercegovini, ispitivan je utjecaj dodatka L-askorbinske kiseline i različitih kupaža na sadržaj polifenola (fenolne kiseline, flavanoli, flavonoli i dihidrohalkoni). Analizirane kupaže sadržavale su 50% sokova Idared/Granny Smith, a preostalih 50% činili su sokovi sorti Tetovke i Paradije u različitim odnosima. Fenolne kiseline su bile dominantno zastupljene u oba tipa soka, dok je sadržaj flavonola bio najniži. Najveći sadržaj flavonola zabilježen je u kupaži mutnog soka (50% Idared + 40 % Paradija + 10 % Tetovka) proizvedenoj sa dodatkom L-askorbinske kiseline.

Ključne riječi: *sadržaj polifenola, L-askorbinska kiselina, kupažiranje, bistri i mutni sokovi*

Summary

This paper focuses on the investigation of polyphenols content in single-cultivar and blended cloudy and clear apple juices, produced with and without the addition of L-ascorbic acid. The effects of L-ascorbic acid addition and different juices blends on the polyphenols content (phenolic acids, flavanols, flavonols and dihydrochalcones) were evaluated in order to improve polyphenolic profiles of apple juices made from predominant commercial apple cultivars grown in Bosnia and Herzegovina. The analysed blends contained 50 % of Idared/Granny Smith juice base and 50 % of Tetovka and Paradija juices in different ratios. In both juices phenolic acids were dominant, while the content of flavonols was the lowest. However, the highest

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Faculty of Agriculture and Food Sciences University of Sarajevo, Bosnia and Herzegovina
[2] NIBIO – Norwegian Institute of Bioeconomy Research, Norway

flavonols content was recorded in blended cloudy juices (50% Idared + 40 % Paradija and 10 % Tetovka juices) produced with added L-ascorbic acid.

Key words: *polyphenols content, L-ascorbic acid, blending, clear and cloudy juices*

INTRODUCTION

Recent scientific studies show strong relationship between the phenolic compounds in apple fruit, which have antioxidant activity, and reduced risk of various diseases (Oszmiański *et al.*, 2007; Barth *et al.*, 2007; Hyson, 2011; Slavin & Lloyd, 2012). Particulary, quercetin glycosides and procyanidins have been linked with strong antioxidant activity in *in vitro* observations (Miguel, 2011). Phenolic profile of an apple varies widely among different parts of the fruit. For instance, quercetin glycosides are present mainly in the skin and dihydrochalcones in seeds, so only small amounts of these compounds are extracted during juice production (Thilen *et al.*, 2005). Despite being abundant in apples, the polyphenolic compounds are known to be lost when apples are processed into juice (Van der Sluis *et al.*, 2002). In conventional apple juice, the antioxidant activity decreases by up to 97% in comparison with fresh apples. Among the many other factors, decreasing of polyphenolic compounds in juice depends on cultivars (Vrhovsek *et al.*, 2004; Begić – Akagić *et al.*, 2011;) treatments (Niu *et al.*, 2010; Vranac *et al.*, 2014) and the oxidative capacity of certain phenolic group (Thilen *et al.*, 2005). According to Grimi *et al.* (2011), during the processing cell wall is being disintegrated and polyphenoloxidase (PPO), the main oxidizing enzyme bounded to cell walls, starts to degrade phenolic compounds, L-ascorbic acid and other constituents of the obtained mash. However, L-ascorbic acid is often added as an antioxidant during the apple juices production to prevent browning, or to replace the vitamin C lost during processing (Mieszczakowska – Frąc *et al.*, 2015). The loss of polyphenols is more evident during the production of clear apple juices. In order to achieve fully clarified juice, which has a brighter colour and a clearer appearance (Ashurst, 2005), raw apple juices are subjected to clarification and filtration operations to remove the pectin substances and fibers (Vaillant *et al.*, 2001; Candrawinata *et al.*, 2012). On the other side, cloudy apple juices have a higher antioxidant capacity, hazy appearance and full bodied sensation, due to the presence of polyphenols, pectin substances and fibers. All of these properties made cloudy apple juices attractive to consumers which led to expanding the market of these products in last years (Braun, 2003; AIJN, 2017). However, the greatest influence on polyphenols content in both cloudy and clear juices have apple cultivar itself.

In the industrial juice processing in Bosnia and Herzegovina cultivars Idared and Granny Smith are dominant. These cultivars are lacking in bioactive compounds. Contrary to them, traditional apple cultivars are valuable sources of desirable genetic (Gaši *et al.*, 2010) and technological characteristics (Begić-Akagić *et al.*, 2007), with significantly higher content of phenols compared to commercial apples (Begić –

Akagić *et al*., 2011). Therefore, traditional bh. cultivars are desirable raw material for improving the quality of juices obtained from commercial cultivars. The purpose of the study was to investigate the influences of L-ascorbic acid addition and blending of apple juices on the content of polyphenolic compounds.

MATERIALS AND METHODS

As a raw material for the single-cultivar cloudy and clear juices production two commercial (Idared and Granny Smith) and two traditional apple cultivars (Paradija and Tetovka) were used. All above listed apple cultivars are part of *ex situ* collection (Voćni rasadnik, d.o.o., Srebrenik, B&H) and were harvested in a technological maturity stage (detected by iodine-starch test). Two types of single-cultivar cloudy and clear juices were produced, in dependence of L-ascorbic acid (AA) addition (+ is for the juices with added AA, - is for the juices without AA), per each apple cultivar. After the inspection, weighting and washing, apples were grinded in electrical grinder. During the apples grinding, 150 mg kg^{-} of the L-ascorbic acid was added gradually. After the grinding, obtained apple mashes were subjected to enzymatic treatment during 30 minutes (Fructozym MA, Erbslöh, Germany). By the completed enzymatic treatment, apple mashes were pressed through inox presser. The obtained raw juices were pasteurized at 78 ºC (total time of heat treatment was cca 15 minutes) and hot juices were filled into hot sterilised glass bottles and cooled. Production of clear juices included depectinization of raw juices by pectolitic enzymes (Fructozym P, Erbslöh, Germany) at 50°C during 60 minutes, clarification by bentonite, gelatine and silica salt addition, decantation, filtration by filter pump (Rover Colombo 12, Italy), pasteurization, filling into sterilized bottles and cooling. Both cloudy and clear juice samples were taken after cooling and kept at -20°C until analysis.

The single-cultivar juices obtained from commercial cultivars Idared and Granny Smith respresented the basis for the creation of juice blends. For the purpose of blending and quality improvement of juices from commercial juices, single-cultivar juices made from Tetovka and Paradija were selected. Cloudy juices made from Paradija cultivar posses significant antioxidative potential due to their high content of polyphenols (Begić – Akagić *et al*., 2011), same as juices from Tetovka. Index of sweetness was calculated for each single-cultivar juices prior to blending. Juice blends (both +AA and –AA) were made per each commercial cultivar juice as two types: the best blend (NK) and blends variants (table 1). The best blend was made that optimal index of sweetness of final blend ranges between 20 and 30. Blend variants were consisted from 50 % juice of. commercial cv. + 50 % ones of traditional cv. in different ratios.

Table 1. Percentual participation of single-cultivar juices in blends

Juice blends	Single-cultivar juices		
	Idared/Granny Smith	Tetovka	Paradija
I	50	30	20
II	50	20	30
III	50	40	10
IV	50	10	40
V	50	25	25
NK* Idared	40	30	30
NK* Granny Smith	20	50	30

NK* - the best blend

Quantification and identification of polyphenolic compounds by HPLC

10 mL of juice was centrifuged (Thermo Scientific SL16 Centrifuge Series, San Jose, CA, USA) at 10 000 rmp for 7 min at 0°C. The supernatant was filtered through the Chromafil AO-45/25 polyamide filter (Macherey-Nagel, Düren, Germany) into a vial. The HPLC analysis were performed with Thermo Scientific Finnigan Surveyor HPLC-PDA (Thermo Scientific, San Jose, CA). The column used for the separation was Pursuit XRs 3 C-18 (5 μm, 4.6 × 150 mm; Agilent Technologies, Santa Clara, CA, USA), maintained at 25 °C. The elution solvents were: (A) - 97 % acetonitrile + 3 % bidestilated water + 0.1 % formic acid, and (B) - 97 % bidestilated water + 3 % acetonitrile + 0.1 % formic acid with the flow rate maintained at 0.6 mL min^{-1}. The gradient method was used, described by Marks *et al.* (2007). According to Bakhshi & Arakawa (2006) the phenolic acids (chlorogenic acid) and flavanols (catechin, epicatechin, procyanidin B1, procyanidin B2) were analyzed at 280 nm; dihydrochalcons (phloridzin) and flavonols (quercetin 3-glucoside; quercetin 3-galactoside and quercetin 3-rhamnoside) were analyzed at 350 nm. Quantification of individual phenolic compounds was achieved according to the concentrations of the corresponding external standard and was expressed in mg L^{-1}.

Statistical analysis

Two-factorial analysis of variance (ANOVA) was conducted (MS Excel 2013) for examinating the influence of L-ascorbic acid addition and blend combination on the content of polyphenols in juices (p=0.05).

RESULTS AND DISCUSSION

The results of analysis of variance revealed existence of significant influences of L-ascorbic addition and blend type on polyphenols content in all evaluated blended juices. Blending of single-cultivar cloudy juices made from Idared cultivar (M) with those obtained from Tetovka and Paradija resulted in improvement of polyphenolic profiles (Figure 1.).

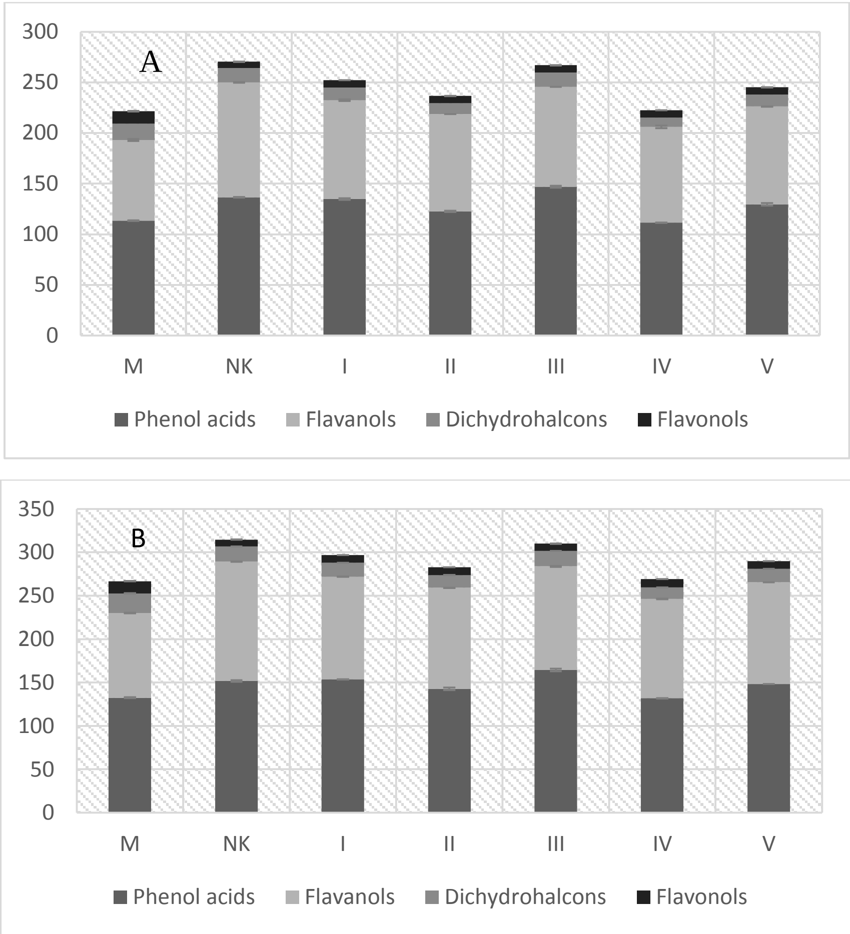


Figure 1. The average polyphenols content (mg L^{-1} ±SD) in Idared based blended cloudy juices without L-ascorbic acid (A) and with treatment (B)

Phenolic acids were the most abundant, followed by flavanols and dichydrohalcons, while flavonols were recorded in lower amounts in analyzed juices produced without the added L-ascorbic acid. These results are in accordance with those reported by Vranac *et al.* (2014) and expected, due to the fact that chlorogenic acid has high water solubility, which eases its transfer from raw material into juice, unlike the other polyphenolic compounds (Van der Sluis *et al.*, 2002). The highest content of phenolic acids (146.9 mg L^{-1}) had blend III (50% Idared+ 40% Tetovka +10% Paradija). The most dominant polyphenolic group in the best blends (Idared 40%; Tetovka 30%; Paradija 30%) were flavanols (113.4 mg L^{-1}), while the content of dichydrohalcons and flavonols were the highest in the single-cultivar Idared juice (16.3 for –AA and 12.2 mg L^{-1} +AA).

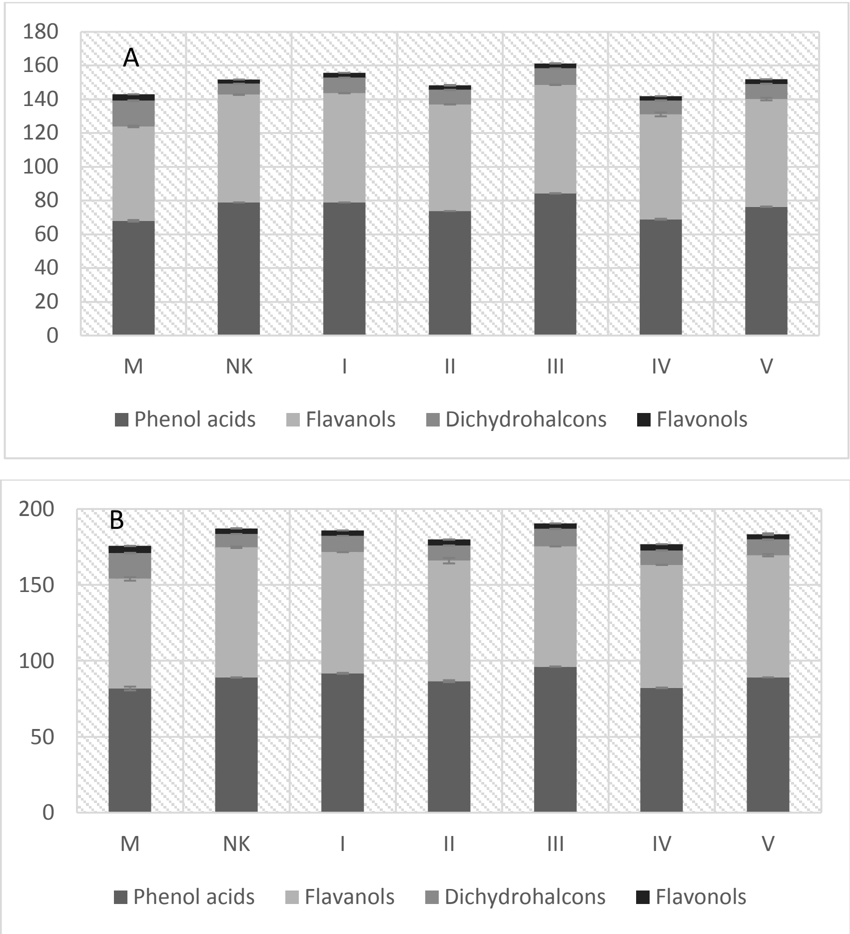


Figure 2. The average polyphenols content (mg L^{-1}±SD) in Idared based blended clear juices without L-ascorbic acid (A) and with treatment (B)

The same order of polyphenol groups was recorded in juices with treatment, but in higher amounts. Obtained results are in agreement with Gliszczynska – Swiglo & Tyrakowska (2003) and Markowski & Plocharski (2005) who found strong correlation between phenolics content and L-ascorbic acid added in clear and cloudy juices. Blending of Tetovka and Paradija cloudy juices with juice originated from cv. Idared resulted in increasing the content of phenolic acids and flavanols in blends, while the similar effect was not achived in terms of dichydrohalcons and flavonols, because the single-cultivar Idared juices initially contained higher quantity of these compounds. Generally, the polyphenolic improvement was more evident for juices produced without L-ascorbic acid, since they originally contained lower polyphenols contents compared to juices with treatment. Moreover, blended clear juices based on

Idared cultivar had the lower content of polyphenols in comparison with cloudy ones (Figure 2.).

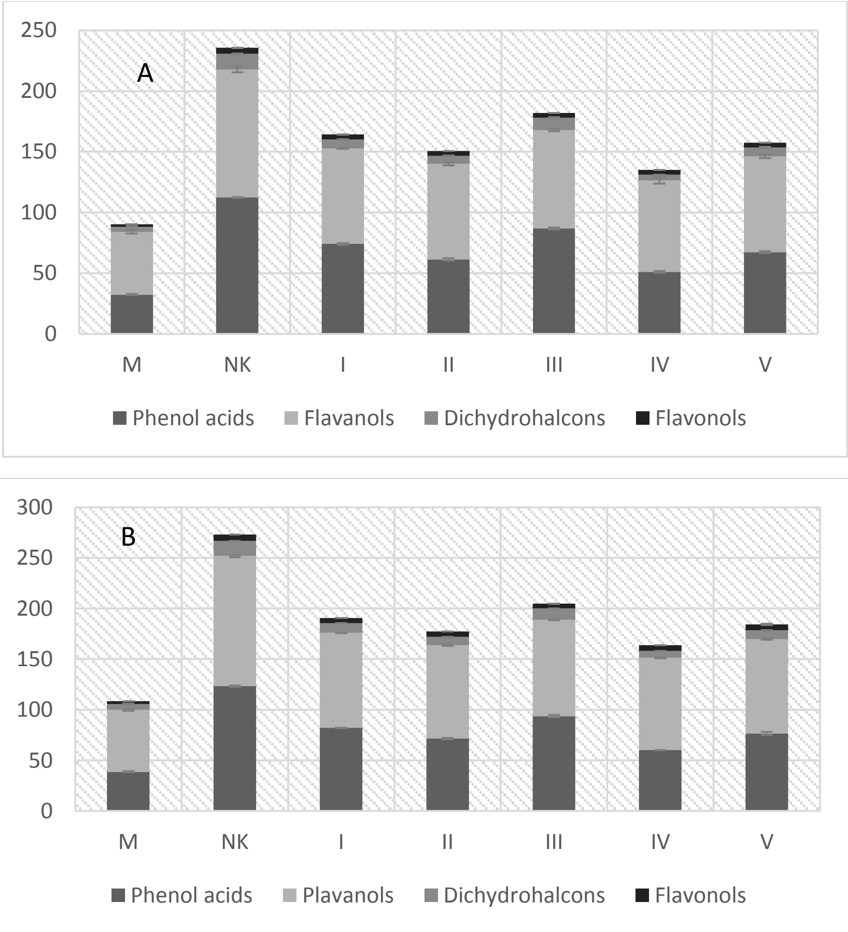


Figure 3. The average polyphenols content (mg L^{-1}±SD) in Granny Smith based blended cloudy juices without L-ascorbic acid (A) and with treatment (B)

The polyphenolic compounds in clear juices were recorded in the same order as in cloudy ones (phenolic acids>flavanols>dihydrochalcons>flavonols). Blending of clear juice made from Idared cultivar (M) with those obtained from Tetovka and Paradija resulted in increasing of the phenolic acids and flavonols content in comparison with single-cultivar juice. Cloudy and especially clear juices, made from Granny Smith cultivar, were not recognised as juices with high content of polyphenolic compounds. Thus, blending them with juices made from traditional cultivars improved polyphenolic profiles of juices both with and without treatment (Figure 3, 4).

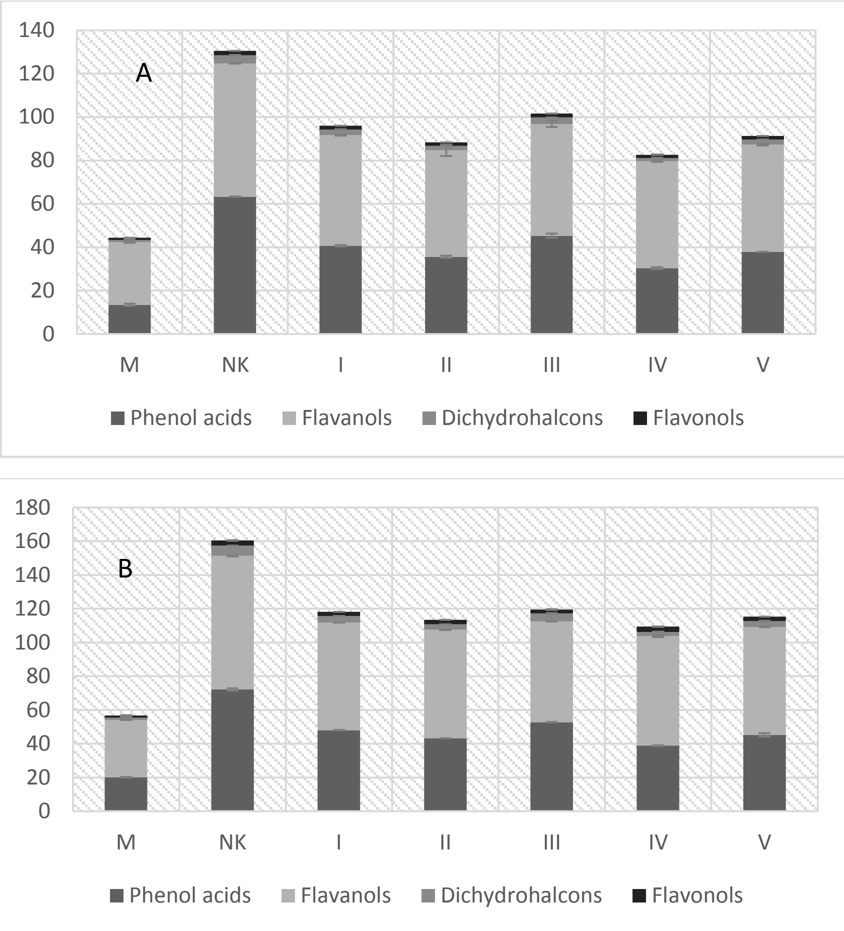


Figure 4. The average polyphenols content (mg L^{-1} ± SD) in Granny Smith based blended clear juices without L-ascorbic acid (A) and with treatment (B)

From presented figures it can be seen that the best blends (Granny Smith 20% +Tetovka 50% + Paradija 30%) had the higher content of polyphenol compounds compared to all the others juices. The most dominant individual polyphenol in cloudy juices (figure 3A, 3B) was chlorogenic acid (the best blends: 112.4 mg L^{-1} –AA; 123.4 mg L^{-1}+AA), followed by flavanols (105.5 mg L^{-1} –AA; 128.4 mg L^{-1} +AA) and dihydrochalcons (12.8 – AA, 14.9 mg L^{-1} +AA respectively) and flavonols (4.9 mg L^{-1} –AA, 6.34 mg L^{-1} +AA). The effect of blending the Granny Smith single-cultivar juices was more expressed in the case of clear juices (Figure 4.). The main reason of that is the fact that clear juices are poorer in the content of bioactive compounds as a result of additional processing operations, such as depectinisation and clarification (Olk *et al.*, 2010; Quoc *et al.*, 2011; Candrawinata *et al.*, 2012). Creation of the best clear Granny Smith based juices blends (Granny Smith 20% + Tetovka 50% +

Paradija 30%) increased the content of phenolic acids for 72.1/78.7% (+AA/-AA), flavanols for 53/57.1% (+AA/-AA), dihydrohalcons 71 (-AA), respectively 77.5% (+AA) and flavonols 51.7 (-AA) and 55.7% (+AA).

CONCLUSIONS

This study demonstrated that blending of single-cultivar juices from commercial cultivars with juices obtained from traditional apple cultivars results in improvement of polyphenolic profiles of final blends. Among quantified polyphenolic compounds in juices (cloudy and clear), produced with and without treatment, the most abundant were chlorogenic acid, followed by flavanols and dihydrochalcons, while the content of flavonols was the lowest. Generally, blending of juices based on cv. Granny Smith had the lower content of polyphenols in comparison with Idared based blends consisted. However, the effect of polyphenols enrichment was more evident for Granny Smith blends, rather than those based on Idared single-cultivar juices. Cloudy and clear juices produced with the added L-ascorbic acid showed higher content of individual polyphenols compared to those made without treatment.

AKNOWLEDGEMENTS

This work was supported by grant from the Programme in Higher Education, Research and Development in the Western Balkans 2011–2014, the Agriculture Sector (HERD/Agriculture): “Evaluation of fruit genetic resources in Bosnia and Herzegovina with the aim of sustainable, commercial utilization”. Authors would also like to thank to Erbslöh Geisenheim AG (Germany) for their kindly support by bestowing their products for juices production.

REFERENCES

AIJN (2017). European Fruit Juice Association, Liquid Fruit Market Report. http://www.aijn.org/pages/main/file.handler?f=AIJNMarketReport2017.pdf

Ashurst P. R. (2005). Chemistry and Technology of Soft Drinks and Fruit Juices. 2nd edn. United Kingdom. Blackwell Publishing.

Bakhshi D., Arakawa O. (2006). Introduction of phenolic compounds biosynthesis with light irradiation in the flesh of red and yellow apples. Journal of Applied Horticulture, 8(2), pp.101-104.

Barth S., Fähndrich C., Bub A., Wartzl B., Will F., Dietrich H., Briviba K. (2007). Cloudy apple juice is more effective than apple polyphenols and an apple juice derived cloud fraction in a rat model of colon carcinogenesis. J. Agric. Food Chem., 55(4), pp.1181–1187.

Begić-Akagić A., Hadžić S., Gaši F., Kurtović M. (2007). Evaluacija tehnoloških svojstava autohtonih genotipova jabuke u B&H. Radovi Poljoprivredno-prehrambenog fakulteta, 57(3). pp. 117-125.

Begić-Akagić A., Spaho N., Oručević S., Drkenda P., Kurtović M., Gaši F., Kopjar M., Piližota V. (2011). Influence of cultivar, storage time, and processing on the phenol content of cloudy apple juice. Croat. J. Food Sci. Technol., 3 (2), pp. 1-8.

Braun G. (2003). World market(s) for apple juice concentrate 2003—Missing apples? Fruit Processing, 13, pp. 422 − 425.

Candrawinata V. I, Blades B. L., Golding J. B., Stathopoulos C. E., Roach P. D. (2012). Effect of clarifiation on the polyphenolic compound content and antioxidant activity of commercial apple juices. International Food Research Journal,19 (3), pp. 1055-1061.

Gaši F., Šimon S., Pojskić N., Kurtović M., Pejić I. (2010). Genetic assessment of apple germplasm in Bosnia and Herzegovina using microsatellite and morphological markers, Scientia Horticulturae, Vol.1 26 (2): pp.164-171.

Gliszczynska-Swiglo A., Tyrakowska B. (2003). Quality of commercial apple juices evaluated on the basis of the polyphenol content and the TEAC antioxidant activity. J. Food Sci., 68, 5, 1844–9.

Grimi N., Mamouni F., Lebovka N., Vorobiev E., Vaxelaire J. (2011). Impact of apple processing modes on extracted juice quality: Pressing assisted by pulsed electric fileds. Journal of Food Engineering. 103, pp.52-61.

Hyson D. (2011). A comprehensive review of apples and apple components and their relationship to human health. Advances in Nutrition. 2(5), pp.408–420.

Markowski J. Plocharski W. (2005). Chemical composition of commercial apple juices in Poland and their antioxidative activity. Proceedings of "European Symposium on Apple Processing", 16–18.03.2005, Rennes, France, 78.

Marks S. C., Mullen W., Croizer A. (2007). Flavonoid and cholorogenic acid profiles of English cider apples. Jornal of Science of Food and Agriculture, 87(4), pp.719-728.

Mieszczakowska-Frąc M., Maria Buczek M., Kruczyńska D., Markowski J. (2015). Cloudy red-fleshed apple juice production and quality. Polish Journal of Natural Sciences 30(1), pp. 59–72.

Miguel M. (2011). Anthocyanins: Antioxidant and/or anti-inflammatory activities. J. Appl. Pharm. Sci.,1(6), pp. 07–15.

Niu S., Xu Z., Fang Y., Zhang L., Yang Y., Liao X., Hu X. (2010). Comparative study on cloudy apple juice qualities from apple slices treated by high pressure carbon dioxide and mild heat. Innovative Food Science and Emerging Technologies.11, pp.91–97.

Olk M., Dietrich H., Ludwig M., Will F. (2010): Impact of variety and vintage on the polyphenol composition of apple juice. Mitteilungen Klosterneuburg, 60, pp.287-296.

Oszmiański J., Wolniak M., Wojdyło A., Wawer I. (2007). Comparative study of polyphenolic content and antiradical activity of cloudy and clear apple juices. J. Sci. Food Agric., 87(4), pp.573–579.

Quoc A. L., Mondor M., Lamarche F., Makhlouf J. (2011). Optimization of electrodialysis with bipolar membranes applied to cloudy apple juice: Minimization of malic acid and sugar losses. Innovative Food Science and Emerging Technologies, 12, pp.45-49.

Slavin J., Lloyd B. (2012). Health benefits of fruit and vegetables. Advances in Nutrition. 3(4), pp.506–516

Thielen C., Will F., Zacharias J., Dietrich H., Jacob H. (2005). Distribution of dihydrochalcones and flavonols in apple tissue and comparison between fruit and juice. Proceedings of "European Symposium on Apple Processing", 16–18.03.2005, Rennes, France, pp.68.

Vaillant F., Millan, A., Dornier M., Decloux M., Reynes M. (2001). Strategy for economical optimisation of the clarifiation of pulpy fruit juices using crossflw microfitration. Journal of Food Engineering 48, pp. 83-90.

Van der Sluis A. A., Dekker M., Skrede G., Jongen W. M. (2002). Activity and concentration of polyphenolic antioxidant in apple juice. Effect of existing production methods. Journal of Agricultural and Food Chemistry 50(25), pp.7211-7219.

Vranac A., Begic-Akagic A., Hudina M., Oražem P., Gaši F., Spaho N., Meland M. (2014). Impact of cultivar and processing stage on the distribution of polyphenols in apples and their juices. Proceedings of 25th International Scientific-Expert Conference of Agriculture and Food Industry – Izmir,Turkey. pp.165-168.

Vrhovsek U., Rigo A., Tonon D., Mattivi F. (2004). Quantitation of polyphenols in different apple varieties. Journal of Agricultural and Food Chemistry, 52, pp. 6532-6538.

SENSORY EVALUATION OF BLENDED CLOUDY APPLE JUICES*

SENZORNA EVALUACIJA KUPAŽIRANIH MUTNIH SOKOVA OD JABUKE

Amila Vranac[1], Asima Akagić[1], Fuad Gaši[1], Nermina Spaho[1], Mirsad Kurtović[1], Mekjell Meland[2]

Original scientific paper

Summary

Sensory quality of cloudy apple juices is highly demanded by consumers, along with its nutritional value and bioactive potential. Sensory attributes of an apple juice are directly dependent on the cultivar used for juice production. In Bosnia and Herzegovina, commercial apple cultivars are predominant in industrial production of cloudy apple juices. These cultivars are often lacking in outstanding sensory characteristics, particularly in aromatic profile and mouth-feel sensation. On the other hand, traditional B&H apple cultivars represent valuable source of desirable nutritional, antioxidative and remarkable sensory properties. The main goal of the study was to improve sensory characteristics of commercial cultivars juices by blending them with juices obtained from traditional cultivars. Therefore, sensory attributes of single-cultivar juices (commercial: Idared, Granny Smith; traditional: Prijedorska zelenika, Funtača, Rebrača, Tetovka, Paradija), and blended juices with commercial single-cultivar juices as a bases were evaluated. Moreover, these juices were judged as samples with and without the added L-ascorbic acid. The obtained results indicated that sensory characteristics of single-cultivar commercial apples juices were significantly improved by blending them with those made from traditional apple cultivars. Particularly odour, aroma, harmonious taste and mouth-feel of blended juices received higher sensory scores in comparison with the single-cultivar commercial apple juices.

Key words: *Sensory properties, apple juices, single-cultivar juices, blending*

Rezime

Senzorni kvalitet mutnih sokova od jabuke svrstava se među najvažnije zahtjeve potrošača, pored zahtjeva za nutritivnim vrijednostima i bioaktivnim potencijalom datih proizvoda. Senzorna svojstva soka od jabuke direktno zavise od osobina

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Faculty of Agriculture and Food Sciences University of Sarajevo, Bosnia and Herzegovina
[2] NIBIO – Norwegian Institute of Bioeconomy Research, Norway

kultivara korištenog za preradu. U industrijskoj proizvodnji sokova u Bosni i Hercegovini, dominantno su zastupljeni komercijalni kultivari jabuka. Međutim, ovi kultivari često ne posjeduju naročito izražena senzorna svojstva, odnosno bogat aromatski profil i naglašenu senzaciju punoće okusa. S druge strane, tradicionalni bh. kultivari jabuka čine vrijedne izvore poželjnih nutritivnih, antioksidativnih i izvanrednih senzornih atributa. Osnovni cilj ovog istraživanja se sastojao u unapređenju senzornih svojstava sokova komercijalnih kultivara putem kupažiranja sa sokovima proizvedenim od tradicionalnih kultivara jabuke. Senzorna evaluacija je uključivala ocjenu svojstva monokomponentnih sokova (komercijalni: Idared i Granny Smith; tradicionalni: Prijedorska zelenika, Funtača, Rebrača, Tetovka i Paradija), te kupažiranih sokova napravljenih na bazi sokova komercijalnih kultivara. Također, sokovi su ocjenjivani kao uzorci sa dodatkom i bez dodatka L-askorbinske kiseline. Rezultati istraživanja ukazuju na to da su senzorna svojstva monokomponentnih sokova komercijalnih kultivara značajno unaprijeđena kroz kupažiranje sa sokovima od tradicionalnih kultivara. Tačnije, evidentno unaprijeđenje postupkom kupažiranja je postignuto u domenu obogaćenja mirisa, arome, harmoničnosti i punoće okusa.

Ključne riječi: *Senzorna svojstva, sokovi od jabuke, monokomponentni sokovi, kupažiranje*

INTRODUCTION

Apple juice is a worldwide known product and highly consumed in Europe, third after orange juice and flavour mixes (AIJN, 2016). Recently, there is an increasing rate of cloudy apple juices consumption because of their high antioxidant activity (Candrawinata *et al*., 2012) and more expressed aromatic profile in comparison to clear juices. Sensory attributes of a cloudy apple juice are directly dependent upon many factors. During the juices production, some of the apples' sensory features can be diminished due to the loss of aroma (Yee Lee *et al*., 2017) and polyphenolic compounds responsible for flavour, colour and mouth-feel impression. The loss can be mitigated by the addition of an antioxidant during the processing, such as L-ascorbic acid (Kolniak-Ostek *et al*., 2013). However, the greatest impact on sensory properties of final juice have technological features of apple cultivar itself. In Bosnia and Herzegovina, among the most cultivated apple cultivars are Idared, Golden Delicious, Pink Lady etc., while in the industrial juice production cultivars Idared and Granny Smith are predominant (Akagić & Vranac, 2017). These cultivars, especially Idared, are characterised by high quantity, availability and storage stability, which is of particular importance for fruit industry. However, these cultivars are often lacking in harmonious taste, aroma and promising bioactive potential. Therefore, in order to meet consumer's demands, enrichment of final products obtained from mentioned cultivars is often necessary. On the other hand, traditional bh. apple cultivars represent valulable source of desirable genetic (Gaši *et al*., 2010, 2013a and 2013b) and technological properties (Begić-Akagić *et al*., 2007; Begić-Akagić *et* *al.*, 2011), primarily high antioxidant activity and outstanding aromatic profile. Nevertheless these cultivars are part of the valuable traditional heritage, their cultivation is non-intensive and sporadic, which results in produced apples quantity that cannot satisfy the industrial needs. Thus, traditional apple cultivars are excellent raw material for blending and enriching of juices based on commercial cultivars. In accordance to that, the main aim of the study was to improve sensory characteristics of commercial juices by blending them with juices obtained from traditional apple cultivars.

MATERIALS AND METHODS

Material

Two commercial (Idared and Granny Smith) and five traditional apple cultivars (Prijedorska zelenika, Funtača, Rebrača, Paradija and Tetovka) were used as a raw material for the single-cultivar cloudy juices production. All above listed apple cultivars are part of *ex situ* collection (Voćni rasadnik, d.o.o., Srebrenik, B&H) and were picked up in a technological maturity stage (detected by iodine-starch test).

Single-cultivar juices production

Per each apple cultivar, two types of single-cultivar cloudy juices were produced, in dependence of L-ascorbic acid (AA) addition (+ is for the juices with added AA, - is for the juices without AA). After the inspection, weighting and washing, apples were grinded in electrical grinder. During the apples grinding, required amount of the L-ascorbic acid was added gradually. According to Krapfenbauer *et al.* (2006), 150 mg kg^{-1} of L-ascorbic acid prevents oxidation of raw material during the processing. After the grinding, obtained apple mashes were subjected to enzymatic treatment during 30 minutes (Fructozym MA, Erbslöh, Germany) with the aim of raw juices' yield, nutritional, antioxidative and aroma improvement. By the completed enzymatic treatment, apple mashes were pressed through inox presser. The obtained raw juices were pasteurized at 78 °C (total time of heat treatment was cca 15 minutes) and hot juices were filled into hot sterilised glass bottles and cooled.

Juices blending

The single-cultivar juices obtained from commercial cultivars Idared and Granny Smith represented the basis for the juice blends. For the purpose of blending and sensory quality improvement of juices from commercial cultivars, single-cultivar juices made from Tetovka and Paradija were selected. According to Alihodzic *et al.* (2013), traditional apple cultivars are characterized by valuable sensory features. Furthermore, cultivars Paradija and Tetovka displays extraordinary flavour in comparison to referent commercial cultivars. Moreover, cloudy juices made from Paradija cultivars have significant antioxidative potential due to their high content of polyphenols (Begić-Akagić *et al.*, 2011). Prior to the juices blending, the content of total soluble solids (°Brix) and titratable acidity (g 100 mL^{-1}) of each single-cultivar juice were determined. On the basis of the total soluble solids and titratable acidity, index of sweetness was calculated. Optimal index of sweetness for apple juices ranges 20-30. Cloudy juice blends (both +AA and –AA) were made per each commercial cultivar juice as two types: the best blend (BB) and blends variants. The best blend was made according to the previous calculation of each juice's percentual total soluble solids and titratable acidity participation so that optimal index of sweetness of final blend ranges between 20 and 30 (Table 1). Blending pattern for the blend variants is shown in table 2.

Table 1. Percentual participation of juices in the final Idared and Granny Smith best blends (%)

Single-cultivar juices	Idared – BB	Granny Smith – BB
Idared	40	-
Granny Smith	-	20
Tetovka	30	50
Paradija	30	30

Table 2. Percentual participation of juices in the final Idared and Granny Smith based blend variants (%)

Single-cultivar juices	Idared or Granny Smith based blend variants				
	I	II	III	IV	V
Idared or Granny Smith	50	50	50	50	50
Tetovka	30	20	40	10	25
Paradija	20	30	10	40	25

Sensory evaluation

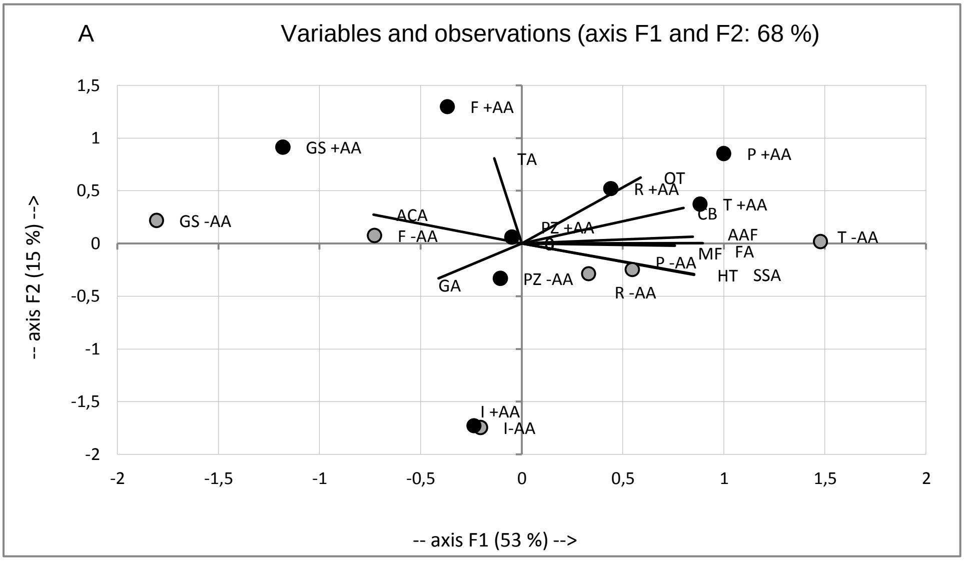
Totally 38 cloudy apple juices (14 single-cultivar and 24 blends) were sensory evaluated by five trained panelists. The samples were evaluated by Quantitative Descriptive Analysis (Spaho, 2017). Grading (1-5, whereas 1 – minimal, 5 – maximal) was carried out for the following sensory parameters: odour (purity, typicality, intensity and overall sensation), colour (purity, browning, typicality and overall sensation), taste (acidity, sweetness, bitter-sweet taste, harmonious taste, astringency, mouth-feel and overall sensation) and aroma (fruity, apple aroma, green, sour aroma, syrup-sweet aroma and overall sensation). Sensory evaluation was carried out in batches with appropriate pauses.

Statistical analysis

Statistical analysis included descriptive analysis, two-factorial (L-ascorbic acid addition and single-cultivar juice/blend variant) analysis of variance (ANOVA), followed by *post hoc* Tukey test (only for overall sensations) and reduced principal component analysis (PCA; StatBox 6.7 Grimmer Soft, France) - due to the large number of factors and their high correlativity.

RESULTS AND DISCUSSION

Evaluation of cloudy single-cultivar apple juices revealed evident dissimilarities between cultivars in their sensory properties. Analysis of variance has shown that differences in evaluated sensory attributes are significantly influenced by cultivar itself, which is particularly visible in sensory properties such as sweetness, acidity, syrup-sweet aroma, sour aroma and colour browning. Furthermore, addition of L-ascorbic acid had an impact on several sensory properties, especially on odour features. Generally, cloudy juices obtained from bh. traditional apple cultivars received higher grades for the majority of sensory attributes (table 3), primarily for those associated with odour, aroma and taste. According to the results of panellists' sensory experience, juices obtained from cultivar Rebrača, as well as Tetovka and Paradija had the outstanding aroma features, such as fruity aroma, apple aroma and aroma of fresh fruit. Juices from cultivar Granny Smith received the lowest grades for majority of sensory attributes, except for acidity. Results of principal component analysis (Fig. 1-A) have shown that mostly astringency, sour aroma and green aroma were separating factors which differed juices among themselves. In such a way, juices made from Granny Smith and Funtača were distinguished from other juices, as expected, due to their expressed acidity and related aromatic properties. On the other side, syrup-sweet aroma and sweetness were mainly ascribed to juices derived from Tetovka and Paradija. When it comes to cloudy juices, its colour along with fuzziness and mouth-feel (full-bodied) are specific properties that distinguish them from clear juices. As Lea *et al*. (1992) stated, polyphenolic compounds in apples are responsible for colour of cloudy juices due to enzymatic browning reactions, and for astringency and full-bodied sensation as well. Juices obtained from Tetovka and Paradija were marked as juices with significant colour browning and mouth-feel, which corresponds to their antioxidant potential.



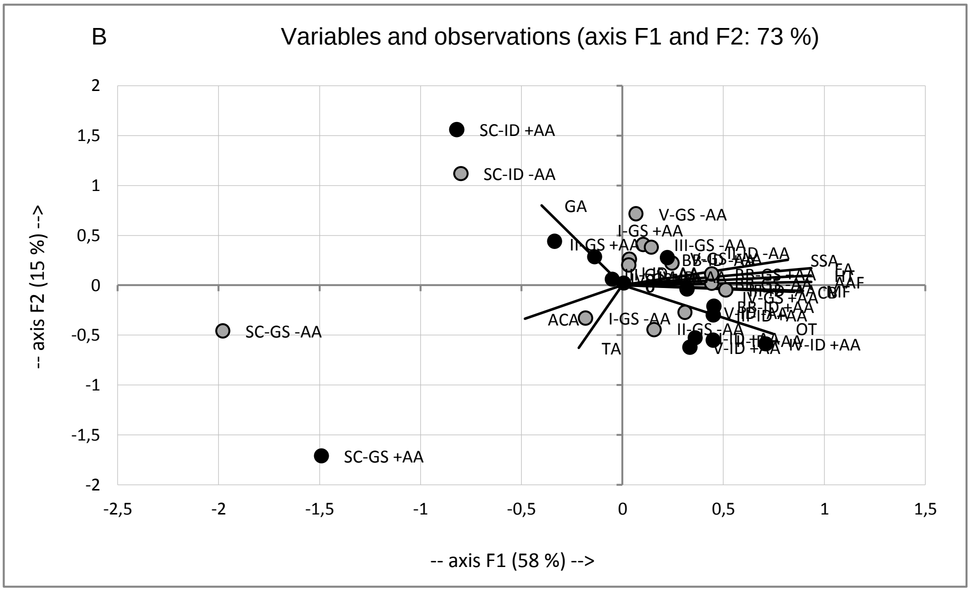


Figure 1. Reduced PCA for single-cultivar (A) and blended juices (B)

Abbreviations: I – Idared, GS – Granny Smith, PZ – Prijedorska zelenika, F – Funtača, R – Rebrača, T – Tetovka, P - Paradija, SC – single-cultivar juice, BB – best blend, I-V (blend variants), AA – L-ascorbic acid ((+)-with AA – black dots; (-)- without AA – grey dots), OT - odour typicality, CB – colour browning, HT - harmonious taste, TA – astringency, MF – mouth feel, FA- fruit aroma, AAF – apple aroma, GA – green aroma, ACA – sour aroma, SSA – syrup-sweet aroma

Blending of juices made from commercial cultivars with those obtained from Tetovka and Paradija resulted in improvement of almost all sensory properties of blends (Fig. 1-B). Principal component analysis has shown that single-cultivar juices from Idared and Granny Smith (both –AA and +AA) significantly differed from their corresponding blends, which is peculiarly visible for Granny Smith juices. Namely, since single-cultivar Granny Smith juices are poor in taste and aroma properties, there was more opportunity to achieve greater improvement in sensory attributes of its blends. However, the PCA results showed that blended juices (both Idared and Granny Smith and both – AA and +AA), were quite homogeneous and similar, which can be due to the short sensory grading scale. Generally, odour, taste and aroma features of single-cultivar juices were improved in blend variants (table 4. and 5). Harmonious taste was reached by blending with Tetovka and Paradija juices, particularly in Granny Smith-based juices which required correction of dominantly expressed acidity. Thus, the greatest effect of blending the Granny Smith juices was index of sweetness optimization. In Idared-based blends, mainly mouth-feel and aromatic properties were improved. Furthermore, predominant green aroma of single-cultivar Idared juices was a bit lowered by their blending. The colour of blends was not significantly improved and the level of colour browning was somewhat increased, due to the more intense browning in Tetovka and Paradija juices. The analysis of variance has shown that the blend variant is significantly influencing the sensory experience, while the addition of L-ascorbic acid in juices is not of particular importance on perceiving the most valuable sensory attributes.

Although the index of sweetness was previously adjusted for the best blends (BB), these type of blends were not the best graded. Generally, the highest scores for the most of sensory attributes had blend variant III (50% of single-cultivar commercial cultivar juice + 40% Tetovka + 10% Paradija), which had an enriching role in sensory properties of Idared juices (- AA and + AA as well). Moreover, blend variant IV (50% of commercial cultivar base + Tetovka 10% and Paradija 40%) was rather highly graded for the many of sensory features in the case of Granny Smith juices (+AA).

Table 3. The average grades for sensory attributes of single-cultivar juices (1-5; ± SD)

Sensory atributtes	Idared		Granny Smith		Prijedorska zelenika		Funtača		Rebrača		Tetovka		Paradija	
	L-ascorbic acid addition													
	-	+	-	+	-	+	-	+	-	+	-	+	-	+
ODOUR														
Purity	2.6 ± 1.3	3 ± 0.7	2.2 ± 1.1	4 ± 0	4 ± 0	4.2 ± 0.5	4.2 ± 0.4	4.2 ± 0.8	4.6 ± 0.5	4.8 ± 0.4	5 ± 0	5 ± 0	4.2 ± 0.4	4.8 ± 0.4
Typicality	2.4 ± 0.9	2.8 ± 0.4	3.2 ± 0.4	4.2 ± 0.4	4 ± 0	4.2 ± 0.4	3.2 ± 0.4	4 ± 0	5 ± 0	4.6 ± 0.5	4.4 ± 0.5	5 ± 0	4.4 ± 0.5	4.8 ± 0.4
Intensity	2.4 ± 0.5	3 ± 0	3 ± 0	3 ± 0	4.2 ± 0.4	4 ± 0	4.4 ± 0.5	4.2 ± 0.4	4.4 ± 0.5	4.4 ± 0.5	5 ± 0	5 ± 0	4.4 ± 0.5	4.8 ± 0.4
Overall sensation	2.2 ± 0.4	2.8 ± 0.4	2.6 ± 0.5	3.4 ± 0.5	4 ± 0	4 ± 0	3.2 ± 0.4	4.2 ± 0.4	4.6 ± 0.5	4.4 ± 0.5	4.6 ± 0.5	5 ± 0	4.4 ± 0.5	4.8 ± 0.4
	d		d		c		a		a		bc		b	
COLOUR														
Purity	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0	4.4 ± 0.5	5 ± 0	5 ± 0	5 ± 0	4.8 ± 0.4	5 ± 0	5 ± 0	5 ± 0
Browning	2 ± 0	1.8 ± 0.4	1.8 ± 0.5	1 ± 0	2 ± 0	2 ± 0	3 ± 0	3.4 ± 0.5	2.3 ± 0.5	4 ± 0.7	5 ± 0	4.6 ± 0.9	4.3 ± 0.5	4.8 ± 0.4
Typicality	4.8 ± 0.4	4.6 ± 0.5	4.8 ± 0.4	4.8 ± 0.4	4.6 ± 0.9	4.4 ± 0.9	4.8 ± 0.4	4 ± 0	4.8 ± 0.4	4 ± 0	3.2 ± 0.4	3.8 ± 0.8	4.4 ± 0.5	3.8 ± 0.4
Overall sensation	4.6 ± 0.9	4.2 ± 0.8	4.2 ± 0.8	4.8 ± 0.4	4.6 ± 0.9	4.4 ± 0.9	4 ± 0.7	4 ± 0	4.8 ± 0.4	4.2 ± 0.4	3.4 ± 0.9	3.8 ± 0.4	4.2 ± 0.8	3.8 ± 0.4
	a		a		a		a		a		a		a	
TASTE														
Acidity	3.2 ± 0.4	3.2 ± 0.4	5 ± 0	5 ± 0	4.2 ± 0.4	4.6 ± 0.5	4.8 ± 0.4	5 ± 0	4.6 ± 0.5	4.4 ± 0.9	3 ± 0	3 ± 0	2 ± 0	2.2 ± 0.4
Sweetness	3.8 ± 0.4	4.2 ± 0.4	1 ± 0	1 ± 0	2.8 ± 0.4	2.4 ± 0.5	2.2 ± 0.4	2 ± 0	3 ± 0	2.6 ± 0.5	5 ± 0	4.2 ± 0.4	3.8 ± 0.4	4.6 ± 0.5
Bitter-sweet	1 ± 0	1.6 ± 0.5	1.2 ± 0.4	1 ± 0	1.8 ± 0.4	1.4 ± 0.5	1.2 ± 0.4	1.6 ± 0.5	1 ± 0	1.4 ± 0.5	1.4 ± 0.5	1.8 ± 0.8	1.8 ± 1.1	2.4 ± 1.3
Harmoniousness	3.4 ± 0.5	3 ± 0	1 ± 0	1.8 ± 0.4	3.2 ± 0.4	3.2 ± 0.4	3 ± 0.7	2.4 ± 0.5	3 ± 0	3.4 ± 0.9	4.6 ± 0.9	3.6 ± 0.5	3.4 ± 0.5	2.8 ± 0.4
Astrigency	1.6 ± 1.3	1.4 ± 0.5	2.2 ± 0.4	2.4 ± 0.5	2 ± 0	2.2 ± 0.4	2.8 ± 0.4	3.2 ± 0.8	1.4 ± 0.5	2.2 ± 0.4	2.2 ± 0.4	2.2 ± 0.4	1.8 ± 0.4	2.6 ± 0.5
Mouth-feel	3 ± 0	3 ± 0	1.8 ± 0.4	2.2 ± 0.4	3.2 ± 0.4	3.2 ± 0.4	3 ± 0	3 ± 0	3.8 ± 0.4	3.2 ± 0.4	4.4 ± 0.9	3.6 ± 0.9	2 ± 0.7	3.6 ± 0.5
Overall sensation	3 ± 0	3 ± 0	1.8 ± 0.4	2 ± 0	3.2 ± 0.8	3.4 ± 0.5	3 ± 0	2.8 ± 0.4	4.2 ± 0.8	3.6 ± 0.5	4.4 ± 0.9	4 ± 0.7	2.8 ± 0.4	3.8 ± 0.8
	a		d		ac		a		c		bc		ac	
AROMA														
Fruity	3.6 ± 0.9	4.2 ± 1.1	2.4 ± 0.5	2.8 ± 0.4	4.8 ± 0.4	4.4 ± 0.5	3.6 ± 0.9	4.2 ± 0.4	5 ± 0	5 ± 0	5 ± 0	4.6 ± 0.5	4.8 ± 0.4	5 ± 0
Apple	3.6 ± 0.5	3.8 ± 0.4	2 ± 0	3.2 ± 0.4	4.2 ± 0.4	4.4 ± 0.5	3.2 ± 0.4	4.2 ± 0.4	5 ± 0	5 ± 0	4.8 ± 0.4	4.2 ± 0.4	4.4 ± 0.9	4.4 ± 0.5
Fruit freshness	4 ± 0	3.8 ± 0.4	3 ± 0.7	4.4 ± 0.5	4 ± 0	4.4 ± 0.5	3.4 ± 0.5	4.2 ± 0.4	4.8 ± 0.4	5 ± 0	4 ± 0.7	4.6 ± 0.5	3.6 ± 0.9	3.6 ± 0.9
Green	3.2 ± 0.4	3.8 ± 1.3	3.2 ± 0.8	2 ± 0	3.8 ± 1.1	3.2 ± 1.1	4.6 ± 0.9	3.6 ± 1.1	3.6 ± 0.5	3.2 ± 1.1	2 ± 0.7	2.6 ± 0.5	2.6 ± 0.5	2 ± 0
SourAcid	3.2 ± 0.4	3.4 ± 0.5	5 ± 0	5 ± 0	4.6 ± 0.5	4.2 ± 0.4	4.8 ± 0.4	5 ± 0	4.6 ± 0.5	4.4 ± 0.9	2.8 ± 0.4	3 ± 0	2.2 ± 0.4	2.2 ± 0.4
Syrup-sweet	4 ± 0	3.4 ± 1.1	1 ± 0	1 ± 0	2.4 ± 0.9	2.2 ± 0.4	2 ± 0	2 ± 0	2.4 ± 0.5	2.4 ± 0.5	5 ± 0	4.4 ± 0.5	3.6 ± 0.5	4.6 ± 0.5
Overall sensation	3.2 ± 0.4	3 ± 0	2 ± 0.7	2.8 ± 0.4	3.8 ± 0.4	3.6 ± 0.5	3 ± 0	2.6 ± 0.5	4.6 ± 0.5	3.6 ± 0.5	4.6 ± 0.9	4 ± 0.7	3.4 ± 0.5	3.4 ± 0.5
	f		e		d		a		c		c		b	

Abbreviations: (+/-) - presence/absence of L-ascorbic acid

Different letters 'a-f ' in rows denote significantly different values among blended juices (Tukey's test, $p < 0.05$)

Table 4. The average grades for sensory attributes of Idared-based blended juices (1-5 ± SD)

	BB		I		II		III		IV		V	
	L-ascorbic acid addition											
	-	+	-	+	-	+	-	+	-	+	-	+
ODOUR												
Purity	5 ± 0	5 ± 0	4.8 ±0.4	4.8 ± 0.4	4.8 ± 0.4	4.8 ± 0.4	5 ± 0	4.8 ± 0.4	5 ± 0	5 ± 0	5 ± 0	4.8 ± 0.4
Typicality	5 ± 0	5 ± 0	4.6 ± 0.5	4.8 ± 0.4	4.4 ± 0.5	4.8 ± 0.4	5 ± 0	4.8 ± 0.4	5 ± 0	5 ± 0	5 ± 0	5 ± 0
Intensity	4.8 ± 0.4	5 ± 0	4 ± 0	4.2 ± 0.4	4.2 ± 0.4	4.2 ± 0.4	4.8 ± 0.4	4.2 ± 0.4	4.2 ± 0.4	4.6 ± 0.5	4.4 ± 0.5	4.8 ± 0.4
Overall sensation	4.8 ± 0.4	5 ± 0	3.8 ± 0.4	4.2 ± 0.4	4.2 ± 0.4	4.4 ± 0.5	4.8 ± 0.4	4.4 ± 0.5	4.4 ± 0.5	5 ± 0	4.6 ± 0.5	4.8 ± 0.4
	a		b		ab		ab		a		a	
COLOUR												
Purity	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0
Browning	3 ± 0.7	4 ± 0	3.2 ± 0.4	3.6 ± 0.5	3.2 ± 0.4	4 ± 0	3.2 ± 0.4	4 ± 0	3.2 ± 0.4	4.4 ± 0.5	3.2 ± 0.4	4 ± 0
Typicality	4.4 ± 0.5	4 ± 0	4.8 ± 0.4	4 ± 0	4.8 ± 0.4	4 ± 0	4.8 ± 0.4	4 ± 0	4.8 ± 0.4	3.8 ± 0.8	4.8 ± 0.4	4.2 ± 0.4
Overall sensation	4.6 ± 0.5	4 ± 0	4.2 ± 0.4	4 ± 0	4.2 ± 0.4	4 ± 0	4.4 ± 0.5	4 ± 0	4.2 ± 0.4	3.6 ± 0.5	4.6 ± 0.5	4 ± 0
TASTE												
Acidity	2.4 ± 0.5	3.6 ± 0.5	2.8 ± 0.4	3.8 ± 0.4	3 ± 0	3.6 ± 0.5	3.2 ± 0.4	3.8 ± 0.4	3.2 ± 0.4	3.8 ± 0.4	4 ± 0	3.8 ± 0.4
Sweetness	4.8 ± 0.4	4.6 ± 0.5	4.2 ± 0.4	4 ± 0	3.2 ± 0.4	4.6 ± 0.5	4 ± 0	4 ± 0	3.4 ± 0.8	4.2 ± 0.4	4 ± 0	3.4 ± 0.5
Bitter-sweet	1.2 ± 0.4	1.6 ± 0.5	1.4 ± 0.8	1.6 ± 0.5	1.2 ± 0.4	2 ± 1	1.2 ± 0.4	1.6 ± 0.5	1.2 ± 0.4	1.6 ± 0.5	1.8 ± 0.4	1.6 ± 0.5
Harmoniousness	3.2 ± 0.4	4.2 ± 0.8	3.6 ± 0.5	4.6 ± 0.5	4.8 ± 0.4	4.8 ± 0.4	5 ± 0	5 ± 0	4.4 ± 0.5	5 ± 0	4.8 ± 0.4	4.4 ± 0.5
Astrigency	1.8 ± 0.4	2 ± 0	2 ± 0	2.2 ± 0.4	1.6 ± 0.5	2.6 ± 0.5	1.6 ± 0.5	2 ± 0	1.2 ± 0.4	1.8 ± 0.4	1.6 ± 0.5	2 ± 0
Mouth-feel	3.2 ± 0.4	4.2 ± 0.4	3.2 ± 0.4	4.2 ± 0.4	4 ± 0	4.4 ± 0.5	4.6 ± 0.5	4.4 ± 0.5	3 ± 1.2	4.8 ± 0.4	4.4 ± 0.5	4.2 ± 0.4
Overall sensation	3.6 ± 0.5	4.4 ± 0.5	3.8 ± 0.4	4.6 ± 0.5	4.2 ± 0.4	4.8 ± 0.4	4.4 ± 0.5	5 ± 0	3.4 ± 0.8	5 ± 0	4.2 ± 0.4	4.2 ± 0.4
AROMA												
Fruity	5 ± 0	5 ±0	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0
Apple	4.2 ± 0.4	5 ± 0	4.8 ± 0.4	5 ± 0	4.8 ± 0.4	4.8 ± 0.4	5 ± 0	5 ± 0	4.8 ± 0.4	5 ± 0	4.6 ± 0.5	5 ± 0
Fruit freshness	4 ± 0	4.6 ± 0.5	4.4 ± 0.8	4.4 ± 0.5	4 ± 0	4.8 ± 0.4	4.4 ± 0.5	4.8 ± 0.4	4 ± 0	4.4 ± 0.5	4.2 ± 0.8	4.6 ± 0.8
Green	2.8 ± 0.4	2.6 ± 0.5	2.8 ± 0.4	2.4 ± 0.5	2.6 ± 0.8	2.6 ± 0.5	2.4 ± 0.8	2.6 ± 0.5	2.8 ± 1.3	2 ± 0	2.4 ± 0.8	2.4 ± 0.5
SourAcid	2.2 ± 0.4	3.2 ± 0.4	3 ± 0	3.4 ± 0.5	3 ± 0	3.2 ± 0.4	3 ± 0	3.8 ± 0.4	3.2 ± 0.4	3.4 ± 05	3.8 ± 0.4	3.8 ± 0.4
Syrup-sweet	4.2 ± 0.4	4.6 ± 0.5	3.6 ± 0.8	4 ± 0	3.6 ± 0.8	4.6 ± 0.5	4.2 ± 0.4	4.2 ± 0.4	3.2 ± 0.4	4.6 ± 0.5	3.8 ± 0.4	3.4 ± 0.5
Overall sensation	3.8 ± 0.4	4.6 ± 0.5	3.8 ± 0.4	4.4 ± 0.5	4 ± 0	4.4 ± 0.5	4.6 ± 0.5	4.8 ± 0.4	3.4 ±0.9	5 ±0	4 ±0	4.2 ±0.4

Abbreviations: BB – The best blend; I-V (blend variants); (+/-) - presence/absence of L-ascorbic acid

Different letters in rows denote significantly different values among blended juices (Tukey's test, $p < 0.05$)

Table 5. The average grades for sensory attributes of Granny Smith-based blended juices (1-5 ± SD)

	BB		I		II		III		IV		V	
	L-ascorbic acid addtion											
	-	+	-	+	-	+	-	+	-	+	-	+
ODOUR												
Purity	4.8 ± 0.4	5 ± 0	4.4 ± 0.5	4 ± 0	5 ± 0	4.6 ± 0.5	4.2 ± 0.4	4.8 ± 0.4	4.2 ± 0.4	5 ± 0	4.6 ± 0.5	4.8 ± 0.4
Typicality	5 ± 0	5 ± 0	4.2 ± 0.4	4 ± 0	5 ± 0	4.4 ± 0.5	4.2 ± 0.4	4.8 ± 0.4	4.2 ± 0.4	4.8 ± 0.4	4.2 ± 0.8	4.6 ± 0.5
Intensity	4.8 ± 0.4	4.2 ± 0.4	3.8 ± 0.4	3.6 ± 0.5	4.8 ± 0.4	4 ± 0	4 ± 0	4.2 ± 0.4	4 ± 0	5 ± 0	4.2 ± 0.4	4.6 ± 0.5
Overall sensation	4.8 ± 0.4	4.8 ± 0.4	3.8 ± 0.4	3.6 ± 0.5	4.8 ± 0.4	4.4 ± 0.5	4 ± 0	4.2 ± 0.4	4.2 ± 0.4	5 ± 0	4.6 ± 0.5	4.6 ± 0.5
	c		b		ac		ab		ac		ac	
COLOUR												
Purity	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0
Browning	3.2 ± 0.4	3.8 ± 0.4	3 ± 0	4 ± 0	3.2 ± 0.4	3.6 ± 0.5	3.2 ± 0.4	3.4 ± 0.5	3.2 ± 0.4	3.6 ± 0.8	3.2 ± 0.4	3.4 ± 0.5
Typicality	4.4 ± 0.5	4 ± 0	4 ± 0	4 ± 0	4 ± 0	4 ± 0	4 ± 0	4 ± 0	4 ± 0	4.2 ± 0.4	4 ± 0	4 ± 0
Overall sensation	4 ± 0	4 ± 0	4 ± 0	4 ±0	4 ± 0	4 ± 0.7	4 ± 0	4 ± 0	4 ± 0	4.2 ± 0.4	4 ± 0	4.2 ± 0.4
TASTE												
Acidity	4 ± 0.7	3.4 ± 0.8	4.8 ± 0.4	4 ± 0	4 ± 0	4 ± 0	3.8 ± 0.4	3.8 ± 0.4	3.6 ± 0.5	3.8 ± 0.4	3.8 ± 0.4	4 ± 0
Sweetness	4.8 ± 0.4	3.8 ± 0.4	3 ± 0	3 ± 0	3.4 ± 0.5	3 ± 0	3.8 ± 0.4	3 ± 0	3.4 ± 0.5	4 ± 0	3.6 ± 0.5	3.8 ± 0.4
Bitter-sweet	1 ± 0	1 ± 0	1.6 ± 0.5	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Harmoniousness	4.8 ± 0.4	4.8 ± 0.4	3 ± 0	3.8 ± 0.4	4.6 ± 0.5	3.6 ± 0.5	4.6 ± 0.5	4 ± 0	4.2 ± 0.4	5 ± 0	4.6 ± 0.5	4.6 ± 0.5
Astrigency	1 ± 0	1.4 ± 0.5	1.8 ± 0.4	1.4 ± 0.5	1.6 ± 0.5	1.4 ± 0.5	1.8 ± 0.4	1.4 ± 0.5	1.8 ± 0.4	1.4 ± 0.5	1.4 ± 0.5	1.4 ± 0.5
Mouth-feel	4.8 ± 0.4	4.8 ± 0.4	4 ± 0.7	3.8 ± 0.4	4.2 ± 0.8	3.4 ± 0.5	4.6 ± 0.5	3.4 ± 0.5	4 ± 0	4.2 ± 0.4	3.8 ± 0.4	4 ± 0
Overall sensation	4.8 ± 0.4	4.8 ± 0.4	4 ± 0.7	4 ± 0	4.2 ± 0.8	3.8 ± 0.4	4.2 ± 0.4	4 ± 0	4.2 ± 0.4	4.8 ± 0.4	4 ± 0	4.4 ± 0.5
	b		a		a		a		ab		ab	
AROMA												
Fruity	4.8 ± 0.4	5 ± 0	4.8 ± 0.4	5 ± 0	4.8 ± 0.4	4.4 ± 0.5	4.8 ± 0.4	4.4 ± 0.5	4.8 ± 0.4	4.8 ± 0.4	5 ± 0	5 ± 0
Apple	4.6 ± 0.5	5 ± 0	4.8 ± 0.4	4.4 ± 0.5	4.8 ± 0.4	4.4 ± 0.5	5 ± 0	4.8 ± 0.4	5 ± 0	5 ± 0	4.8 ± 0.4	4.8 ± 0.4
Fruit freshness	4.6 ± 0.5	4 ± 0	5 ± 0	4.8 ± 0.4	5 ± 0	4.6 ± 0.5	4.6 ± 0.5	4.6 ± 0.8	4.8 ± 0.4	4.8 ± 0.4	4.8 ± 0.4	4.6 ± 0.5
Green	2.2 ± 0.4	2.8 ± 0.4	2.6 ±0.5	3 ± 1	2.4 ± 0.5	3.4 ± 0.5	3.2 ± 0.8	2.8 ± 0.4	3 ± 0.7	2.6 ± 0.5	3.2 ± 0.8	2.8 ± 0.4
SourAcid	3.8 ± 0.4	4 ± 0.7	4.6 ± 0.5	4.4 ± 0.5	4 ± 0	4.4 ± 0.5	4 ± 0	4 ± 0	4 ± 0	4.4 ± 0.5	3.8 ± 0.4	4 ± 0
Syrup-sweet	4.4 ± 0.8	3.8 ± 0.4	3.2 ± 1.1	2.4 ± 0.5	2.8 ± 0.4	2.4 ± 0.5	3.8 ± 0.4	3.2 ± 0.4	3.6 ± 0.5	4 ± 0.7	3.6 ± 0.5	4 ± 0
Overall sensation	4.4 ± 0.5	4.6 ± 0.5	4 ± 0.7	4 ± 0	4 ± 0.7	3.8 ± 0.4	4 ± 0	4.2 ± 0.4	4.4 ± 0.5	5 ±0	4 ± 0	4.6 ± 0.5
	ab		b		b		ab		a		ab	

Abbreviations: BB – The best blend; I-V (blend variants); (+/-) - presence/absence of L-ascorbic acid

Different letters 'a-c' in rows denote significantly different values among blended juices (Tukey's test, $p < 0.05$)

CONCLUSIONS

On the basis of presented results, it can be concluded that blending of single-cultivar juices from commercial cultivars with juices obtained from traditional apple cultivars results in their taste and aroma enrichment and overall improvement. Primarily, by blending of juices in different ratios, odour and aroma properties are significantly improved, and taste correction is evident as well. Likewise, by the addition of L-ascorbic acid during the juice production, some of sensory properties of final products can be preserved. Generaly, overall improvement of sensory properties is observed for blend variant consisted from 50% of commercial cultivar juices base with 40% of Tetovka and 10% of Paradija juice. Moreover, single-cultivar juices from Rebrača should be included in future studies due to it's excellent sensory properties, as noticed in this research. The results of the conducted study are showing that bh. traditional apple cultivars are indeed promising and valuable raw material which requires greater attention in commercial industrial utilization.

AKNOWLEDGEMENTS

This work was supported by grant from the Programme in Higher Education, Research and Development in the Western Balkans 2010–2013, the Agriculture Sector (HERD/Agriculture): “Evaluation of fruit genetic resources in Bosnia and Herzegovina with the aim of sustainable, commercial utilization”. Authors would also like to thank to Erbslöh Geisenheim AG (Germany) for their kindly support by bestowing their products for juices production.

REFERENCES

Akagić, A. & Vranac, A. (2017): Svojstva voća za proizvodnju sokova. Poljoprivredno-prehrambeni fakultet Univerziteta u Sarajevu. Sarajevo, B&H.

AIJN (2016): European Fruit Juice Association – Liquid Fruit Market Report. (http://viewer.zmags.com/publication/1d1b0aa7#/1d1b0aa7/1).

Alihodzic, A., Gasi, F., Drkenda, P., Begic-Akagic A., Meland, M., Kurtovic, M., Spaho, N. (2013): Autochthonous fruit varieties as a basis of B&H food industry sustainable competitive advantages. EFFoST Annual Meeting. Bio-Based Technologies in the Context of European Food Innovation Systems. Bologna, Italy.

Begić-Akagić, A., Spaho, N., Oručević, S., Drkenda, P., Kurtović, M., Gaši, F., Kopjar, M., Piližota, V. (2011): Influence of cultivar, storage time, and processing on the phenol content of cloudy apple juice. Croat. J. Food Sci. Technol., 3 (2), pp. 1-8.

Begić-Akagić, A., Hadžić, S., Gaši, F., Kurtović, M. (2007): Evaluacija tehnoloških svojstava autohtonih genotipova jabuke u B&H. Radovi Poljoprivredno-prehrambenog fakulteta, 57(3). pp. 117-125.

Candrawinata, V. I., Blades, J. B., Stathopoulos, C. E., Roach, P. D. (2012): Effect of clarification on the polyphenolic compound content and antioxidant activity of commercial juices. International Food Research Journal 19 (3). pp. 1055-1061.

Gaši, F., Žulj Mihaljević, M., Šimon, S., Grahić, J., Pojskić, N., Kurtović M., Nikolić, D. and Pejić, I. (2013a): Genetic structure of apple accessions maintained *ex situ* in Bosnia and Herzegovina examined by microsatalite markers. Genetika, Vol. 45 (2). pp. 467-478.

Gaši, F., Šimon, S., Pojskić, N., Kurtović M., Pejić, I., Melan, M., Kaiser, C. (2013b): Evaluation of Apple (*Malus x domestica*) Genetic Resources in Bosnia and Herzegovina Using Microsatellite Markers. HortScience, Vol. 48. pp. 13-21.

Gaši, F., Šimon, S., Pojskić, N., Kurtović M., Pejić, I. (2010): Genetic assessment of apple germplasm in Bosnia and Herzegovina using microsatellite and morphological markers, Scientia Horticulturae, Vol.1 26 (2): pp. 164-171.

Krapfenbauer, G., Kinner, M., Gössinger, M., Schonlechner, R., Berghofer, E. (2006): Effect of Thermal Treatment on the Quality of Cloudy Apple Juice. J Agric Food Chem. 26:54 (15). pp. 5453-60.

Spaho, N. (2017): Senzorna analiza voćnih sokova In: Tehnologija sokova i nektara (eds. Akagić, A. & Spaho, N). Poljoprivredno-prehrambeni fakultet Univerziteta u Sarajevu. Sarajevo, B&H. pp. 224-297.

Yee Lee, P., Kebede, B. T., Lusk, K., Mirosa, M., Oey, I. (2017): Investigating consumers' perception of apple juice as affected by novel and conventional processing technologies. International Journal of Food Science and Technology. pp. 1-8.

EFFECT OF HIGH HYDROSTATIC PRESSURE ON THE VOLATILE COMPOUNDS IN WINE*

UTJECAJ VISOKOG HIDROSTATSKOG TLAKA NA HLAPIVE SPOJEVE U VINU

Marina Tomašević[1], Katarina Lukić[1], Tomislav Bosiljkov[1], Karla Kelšin[1], Natka Ćurko[1], Karin Kovačević Ganić[1]

Original scientific paper

Summary

Application of high hydrostatic pressure (HHP) as innovative technology for food preservation and processing has increased substantially during the last decade. Recently, HHP has been identified as potential alternative process for microbial preservation of wine, as well as wine aging accelerator throughout modifying wine physicochemical and sensorial characteristics, primarily phenolic composition, color and astringency intensity. Due to the lack of information about its influence on aroma composition, the aim of this paper was to study the effect of HHP on volatile aroma compounds of young white and red wines (*Vitis vinifera* L. Graševina and Cabernet Sauvignon). Wines were pressurized at 200, 400 and 600 MPa for 5, 15 and 25 min and analyzed immediately after treatment. Volatile aroma compounds were identified and quantified by solid-phase microextraction coupled with gas chromatography-mass spectrometry (SPME-GC/MS). Applied treatments resulted in slight changes in concentrations of aroma compounds, primarily decrease of esters in both, white and red wine. But, in most cases the observed differences were not significant. Obtained results suggest that HHP could be potentially used as an alternative process to sulfur dioxide addition, primarily to inactivate bacteria and yeasts without causing quality changes.

Key words: *high hydrostatic pressure, aroma compounds, wine, GC/MS*

Sažetak

Primjena visokog hidrostatskog tlaka (HHP) kao inovativne tehnologije u konzerviranju i preradi hrane u zadnjem je desetljeću u značajnom porastu. U posljednje vrijeme HHP tehnologija prepoznata je kao potencijalna, alternativna metoda za mikrobiološko konzerviranje vina te također, kao metoda čijom bi se

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina

[1] Prehrambeno-biotehnološki fakultet, Sveučilište u Zagrebu/ Faculty of Food Technology and Biotechnology, University of Zagreb

primjenom kroz modifikaciju fizikalno-kemijskih i senzorskih karakteristika, prvenstveno polifenolnog sastava, intenziteta boje i trpkoće ubrzao proces starenja vina. Uslijed nedostatka informacija o utjecaju navedene tehnologije na sastav arome, cilj ovog rada bio je istražiti utjecaj HHP tretmana na hlapive komponente mladih bijelih i crnih vina (*Vitis vinifera* L. Graševina and Cabernet Sauvignon). Uzorci vina tretirani su pri tlakovima od 200, 400 i 600 MPa u trajanju od 5, 15 i 25 minuta te analizirani odmah po završetku tretmana. Spojevi arome identificirani su i kvantificirani primjenom plinske kromatografije uz masenu detektciju (GC/MS) uz prethodnu mikroekstrakciju na čvrstoj fazi (SPME tehnika). Primijenjeni HHP tretmani rezultirali su blagim promjenama u koncentracijama spojeva arome, prvenstveno smanjenjem estera u uzorcima bijelog i crnog vina. U većini provedenih tretmana uočene razlike nisu bile značajne. Dobiveni rezultati impliciraju kako bi se HHP tehnika mogla koristiti kao alternativni postupak dodavanju sumporovog dioksida, prvenstveno u cilju inaktivacije bakterija i kvasaca, a da se pritom ne uzrokuju promjene u kvaliteti vina.

Ključne riječi: *visoki hidrostatski tlak, spojevi arome, vino, GC/MS*

INTRODUCTION

The application of high hydrostatic pressure (HHP) in wine technology has shown a great potential during last few years since it is known that wine is very sensitive to temperature increases and it cannot be treated by heat due to its negative influence on aroma, taste and color properties. At the same time, application of HHP, as non-thermal technology, does not result in a significant increase of temperature of wine, and thereby provide preservation of physicochemical properties and overall quality of treated wine. Previous research, regarding the application of HHP treatment on wine, are mainly focused on its influence on the inactivation of undesired microorganisms (Buzrul, 2012; Briones – Labarca *et al.*, 2017). In addition to microbial inactivation, HHP has shown the effect of enhancing some properties without affecting important quality characteristics such as color, pH and turbidity (Briones – Labarca *et al.*, 2017). Recent studies have shown that HHP can also be successfully applied as a technique for increasing the extraction of polyphenolic compounds from grapes and improving the overall quality of wine (Morata *et al.*, 2015), as well as in accelerating the aging process of wine (Sun *et al.*, 2016). It has also been found that the application of HHP does not significantly affect the basic physicochemical characteristics of wine, immediately after processing (Mok *et al.*, 2006). Moreover, changes in physicochemical and sensory properties of wine are only visible in the case of extreme HHP treatment parameters (650 MPa for 1 and 2 hours) (Buzrul, 2012; Tao *et al.*, 2012) and after a certain period of storage through stimulation of Maillard's reactions and polymerization reactions of polyphenolic compounds (Santos *et al.*, 2013; Santos *et al.*, 2015; Santos *et al.*, 2016). Despite mentioned studies, most of the conducted researches regarding the application of HHP technique on wine are related on its effect on the inactivation of undesired microorganisms. There is a lack of information about its influence on chemical changes in wine, primarily aroma and polyphenol compounds. As reported by Tao *et al.* (2012) the chemical reactions influenced by HHP are expected to develop during aging according to Le Chatelier's principle, where the volume reducing during HHP processing could change of equilibrium of chemical reactions (Norton and Sun, 2008). Given the above, HHP potentially could affect chemical reactions in wine and accelerate wine aging process. It is previously demonstrated that applied HHP treatment (350 MPa during 10 min) did not resulted in sensory different wines in comparison to non-treated ones (Mok *et al.*, 2006). Similarly, Puig *et al*. (2003) found no changes in physicochemical properties after HHP treatment (500 MPa for 5 min). However, combination of HHP treatment along with higher temperature resulted in condensation reactions of anthocyanins (Corrales *et al*., 2008). To our best knowledge, there is only one research regarding the influence of HHP on volatile aroma compounds: Morata *et al.* (2012) investigated the influence of HHP treatment (100 MPa for 24 h) on wines contaminated with *Brettanomyces bruxelensis* yeast where only small differences were observed in concentration of higher alcohols and esters after applied HHP. Since HHP represents potent technique in a view of controlling microbial population in wine, and consequently could result in reduced sulfur dioxide additions during wine production, the aim of this paper was to evaluate the influence of HHP processing parameters on the volatile aroma compounds, as one of the most important quality parameter, in white and red wines, *Vitis vinifera* L. Graševina and Cabernet Sauvignon.

MATERIAL AND METHODS

Wine samples

The research was conducted on one young quality dry white wine, variety Graševina (Erdutski vinogradi, Erdut, Croatia) and one young quality dry red wine, variety Cabernet Sauvignon (Erdutski vinogradi, Erdut, Croatia); all vintage 2016. Physicochemical properties of Graševina were: 11.4 vol %, total acidity (as tartaric acid) 5.1 g/L, volatile acidity (as acetic acid) 0.31 g/L, pH 3.37, reducing sugars 2.8 g/L, total extract 20.2 g/L, malic acid 1.2 g/L, while those of Cabernet Sauvignon were: 13.1 vol %, total acidity (as tartaric acid) 5.3 g/L, volatile acidity 0.61 g/L, pH 3.46, reducing sugars 4.1 g/L, total extract 31.7 g/L, lactic acid 1.3 g/L.

Chemicals

Ethanol was HPLC grade and purchased from J. T. Baker (Deventer, Netherlands), sodium chloride p.a. was purchased from Carlo Erba (Val de Reuil, Spain), while the aroma reference standards were purchased from Sigma Aldrich (St. Louis, USA).

High hydrostatic pressure treatment

A high hydrostatic pressure system FPG7100 (Stansted Fluid Power, Harlow, UK) was used for pressurization. The 100 mL of wine was poured into plastic bottle, sealed and placed in the working vessel with maximum capacity of 2000 mL. To assess the possible effects of the HHP treatment experimental test included variations of pressures (200, 400 and 600 MPa) and processing time (5, 15 and 25 min). All the treatments were conducted at room temperature (25 °C) and in triplicate. Control sample represents the wine sample not exposed to the HHP treatment.

Volatile compounds analysis

Volatile compounds were extracted by solid-phase microextraction (SPME) and analyzed by gas chromatography coupled with mass spectrometry (GC/MS) using an Agilent Gas Chromatography 6890 series equipped with an Agilent 5973 Inert mass selective detector (Agilent Technologies, Santa Clara, USA) according to the method in detail described by Tomašević *et al.* (2017).

Data analysis

Significant differences between samples for each of the constituents was determined by one-way analysis of variance (ANOVA) using the Statistica V.10 software (StatSoft Inc., Tulsa, USA). Tukey's honestly significant difference (HSD) test ($p<0.05$) was used for comparison when samples differed significantly after ANOVA was performed. The principal component analysis (PCA) was performed on the correlation matrix using the attributes of aroma compounds analysis in order to examine any possible grouping of samples by different applied treatments.

RESULTS AND DISCUSSION

Different aroma compounds were identified and quantified in analyzed wines, where the esters represent the largest group, followed by higher alcohols, volatile fatty acids, terpene and aldehyde. Concentrations determined in control Graševina wine, as well as in HHP treated ones, are presented in Table 1. As can be seen in table, slight changes occurred after the HHP treatment. Primarily, most of the esters lightly decreased, as well as terpene linalool, volatile fatty acids and benzaldehyde. On the other hand, increase in concentration of amyl alcohol was observed in most of the treated wines, while concentration of 1-hexanol increased at higher pressure (600 MPa). Regarding treatment duration, longer treatment resulted in lower concentration of most analyzed compounds (esters, terpene and aldehyde) except the most of higher alcohols which were determined in slightly higher concentrations in treated wines.
Similar trend was observed after red wine Cabernet Sauvignon treatment: decrease of esters, terpene linalool and benzaldehyde and volatile fatty acids, while concentration of previously mentioned higher alcohols (amyl alcohol and 1-hexanol) slightly increased. Despite similarity with trend found in the case of white wine, after HHP treatment of Cabernet Sauvignon more pronounced changes occurred. For example, concentrations of ethyl hexanoate, ethyl octanoate and especially ethyl decanoate

significantly decreased after applied pressures. The most pronounced changes occurred after pressurization with pressure of 600 MPa. Also, as previously stated, longer treatment duration resulted in higher loss of observed volatiles. Generally, esters represent one of the most important wine aroma groups, contributing to the fresh and fruity characters of wines and they are very sensitive to either thermal treatments or freezing (Lambert *et al.*, 1999). Morata *et al.* (2012) investigated influence of HHP treatment (100 MPa during 24 h) on the inactivation of spoilage *Brettanomyces* yeast in red wine, and beside the antimicrobial effect, they examined the influence of this process on the concentrations of esters and found no significant differences in concentrations of ethyl acetate and ethyl lactate in treated wines, compared to control ones.

In the present literature data, contradictory results of HHP effect on volatile composition could be observed. For example, Briones-Labarca *et al.* (2017) found no change in several aroma compounds (aldehydes, ethyl acetate, propanol, *i*-butanol, butanol and *i*-amyl alcohol) after HHP treatment, while Santos *et al.* (2015) found quite significant changes in aroma composition of treated wine samples. Latter authors found that HHP treatment had a large impact on the volatile composition of both white and red wines. Authors investigated the long-term effect (9 months after treatment) of HHP treatments (425 and 500 MPa during 5 min) on the volatile compounds of red and white native Portugal grape wines and concluded that pressurized wines had higher concentrations of acetals, ketones, furans and aldehydes, compounds that are usually characteristic for wine aging aroma. Also, these authors suggest that HHP treatment accelerate Maillard reaction and oxidation of alcohols and fatty acids, producing the wines with characteristics of faster aging.

Projection of analyzed sensory variables and the distribution of control and HPU treated Graševina wines in the two-dimensional coordinate system defined by first two variables explaining 84.26 % of the total variance is shown in Figure 1 First variable (PC 1) showed strong negative correlation with the content of majority of the analyzed volatile compounds: ethyl acetate (-0.99), *i*-butyl acetate (-0.90), ethyl butyrate (-0.97), *i*-amyl acetate (-0.96), ethyl hexanoate (-0.90), hexyl acetate (-0.96), *cis*-3-hexenol (-0.76), ethyl octanoate (-0.89), benzaldehyde (-0.92), linalool (-0.88), ethyl decanoate (-0.93), 2-phenylethyl acetate (-0.72), diethyl succinate (-0.78), hexanoic acid (-0.88), octanoic acid (-0.73), decanoic acid (-0.89) and on the other side, was highly positively correlated with volatile *i*-amyl acohol (0.70), 1-hexanol (0.82) and 2-phenylethanol (0.95). Furthermore, the second principal component showed a slight negative correlation with most of the analyzed volatile compounds. Control Graševina wine sample (Control W) was placed on the left side of first factorial plane (in the third quadrant) and was displaced from rest of the treated wines due to higher concentrations of aroma compounds which negatively correlate with both first and second factorial plane. The distribution of HHP treated wine samples in the coordinate system indicate clear separation of treated wine samples according to the height of the applied pressure and the treatment duration. In accordance to the mentioned, wine sample pressurized by 200 MPa during 5 minutes was placed within third quadrant and according to that, characterized by higher concentrations of acetate esters and fatty acids in comparison with other HHP treatments. Wine samples pressurized by 200 MPa during 15 and 25 minutes as well as the sample pressurised by 400 MPa during 5 minutes were positioned in second quadrant and characterized by higher concentrations of volatile compounds which correlate negatively with PC1 and positively with PC 2. Furthermore, samples pressurized by 400 and 600 MPa during 5, 15 and 25 minutes are positioned in first and fourth quadrant and are characterized by more significant content of higher alcohols (2-phenylethanol, amyl alcohol, 1-hexanol).

Table 1. Concentration of volatile compounds in control and HHP treated white wines Graševina

	Control	200 MPa			400 MPa			600 MPa		
		5 min	15 min	25 min	5 min	25 min	15 min	5 min	15 min	25 min
Esters (mg/L)										
ethyl acetate	26,90±0,48^{b}	26,29±0,52^{ab}	26,06±0,41^{ab}	26,03±0,21^{ab}	26,05±0,08^{ab}	25,62±0,26^{ab}	25,51±1,19^{a}	25,50±0,24^{a}	25,27±0,45^{a}	25,03±0,06^{a}
ethyl butyrate	0,74±0,04^{b}	0,72±0,01^{ab}	0,72±0,03^{ab}	0,72±0,04^{ab}	0,71±0,04^{ab}	0,70±0,01^{ab}	0,69±0,01^{ab}	0,68±0,03^{ab}	0,68±0,02^{ab}	0,67±0,01^{a}
ethyl hexanoate	0,89±0,12^{a}	0,88±0,07^{a}	0,87±0,06^{a}	0,86±0,03^{a}	0,86±0,03^{a}	0,85±0,03^{a}	0,84±0,02^{a}	0,78±0,03^{a}	0,78±0,00^{a}	0,75±0,06^{a}
ethyl octanoate	0,59±0,01^{b}	0,55±0,08^{ab}	0,51±0,05^{ab}	0,51±0,03^{ab}	0,50±0,04^{ab}	0,49±0,08^{ab}	0,47±0,06^{a}	0,48±0,06^{ab}	0,47±0,05^{ab}	0,48±0,01^{ab}
ethyl decanoate	0,27±0,01^{c}	0,26±0,01^{bc}	0,25±0,01^{abc}	0,24±0,01^{abc}	0,22±0,01^{abc}	0,21±0,01^{abc}	0,23±0,00^{abc}	0,20±0,06^{ab}	0,19±0,06^{a}	0,19±0,02^{a}
diethyl succinate	0,24±0,03^{c}	0,20±0,02^{ab}	0,19±0,01^{a}	0,18±0,01^{a}	0,18±0,02^{a}	0,19±0,01^{ab}	0,19±0,01^{a}	0,19±0,02^{a}	0,18±0,02^{a}	0,18±0,03^{a}
i-butyl acetate	0,11±0,00^{b}	0,11±0,01^{b}	0,11±0,01^{ab}	0,10±0,00^{ab}	0,11±0,01^{ab}	0,11±0,01^{ab}	0,10±0,01^{ab}	0,10±0,00^{ab}	0,10±0,01^{ab}	0,09±0,00^{a}
i-amyl acetate	3,61±0,02^{a}	3,58±0,05^{a}	3,56±0,01^{a}	3,57±0,04^{a}	3,55±0,01^{a}	3,53±0,05^{a}	3,50±0,18^{a}	3,52±0,04^{a}	3,50±0,06^{a}	3,50±0,01^{a}
hexyl acetate	0,41±0,03^{d}	0,36±0,01^{bc}	0,34±0,04^{ab}	0,33±0,02^{ab}	0,34±0,00^{ab}	0,33±0,01^{ab}	0,31±0,01^{a}	0,32±0,01^{ab}	0,31±0,01^{a}	0,30±0,01^{a}
2-phenylethyl acetate	0,28±0,01^{a}	0,27±0,02^{a}	0,26±0,01^{a}	0,25±0,03^{a}	0,27±0,01^{a}	0,24±0,01^{a}	0,25±0,01^{a}	0,25±0,02^{a}	0,26±0,02^{a}	0,24±0,01^{a}
Higher alcohols (mg/l)										
amyl alcohol	57,17±2,45^{a}	58,85±0,49^{ab}	59,63±2,56^{ab}	57,53±0,06^{a}	58,92±0,32^{ab}	59,04±0,76^{ab}	59,26±0,97^{ab}	57,91±0,53^{a}	60,39±3,13^{ab}	62,82±3,44^{b}
2-phenylethanol	4,39±0,60^{a}	4,36±0,06^{a}	4,57±0,04^{a}	4,51±0,05^{a}	4,62±0,56^{a}	4,67±0,17^{a}	4,65±0,67^{a}	4,71±0,60^{a}	4,84±0,01^{a}	4,95±0,04^{a}
1-hexanol	1,15±0,02^{a}	1,15±0,01^{a}	1,15±0,03^{a}	1,16±0,01^{a}	1,16±0,02^{a}	1,16±0,04^{a}	1,17±0,02^{a}	1,17±0,03^{ab}	1,20±0,04^{ab}	1,24±0,07^{b}
cis-3-hexenol	0,12±0,00^{a}	0,12±0,00^{a}	0,12±0,01^{a}	0,11±0,00^{a}	0,11±0,00^{a}	0,11±0,00^{a}	0,11±0,00^{a}	0,10±0,01^{a}	0,11±0,01^{a}	0,11±0,00^{a}
Fatty acids (mg/l)										
hexanoic acid	3,71±0,16^{a}	3,69±0,07^{a}	3,60±0,15^{a}	3,53±0,09^{a}	3,57±0,35^{a}	3,53±0,28^{a}	3,58±0,11^{a}	3,57±0,04^{a}	3,50±0,13^{a}	3,50±0,04^{a}
octanoic acid	10,90±0,72^{a}	10,73±0,36^{a}	10,70±0,05^{a}	10,69±0,09^{a}	10,79±1,27^{a}	10,72±0,33^{a}	10,71±0,30^{a}	10,62±0,09^{a}	10,70±0,02^{a}	10,67±0,12^{a}
decanoic acid	2,25±0,00^{a}	2,22±0,07^{a}	2,16±0,49^{a}	2,08±0,25^{a}	2,04±0,21^{a}	2,05±0,05^{a}	2,01±0,09^{a}	2,05±0,13^{a}	2,01±0,10^{a}	2,03±0,18^{a}
Terpenes (µg/l)										
linalool	4,85±0,07^{a}	4,79±0,09^{a}	4,78±0,03^{a}	4,75±0,20^{a}	4,78±0,07^{a}	4,82±0,72^{a}	4,72±0,02^{a}	4,70±0,06^{a}	4,66±0,04^{a}	4,64±0,05^{a}
Aldehydes (µg/l)										
benzaldehyde	56,17±1,01^{c}	54,31±1,33^{bc}	54,08±2,18^{bc}	54,78±2,38^{c}	53,90±3,92^{bc}	49,48±0,74^{ab}	48,41±1,61^{a}	47,62±1,85^{a}	46,49±3,99^{a}	45,54±1,36^{a}

Data presented as average value of three analytical repetitions (N=3) ± standard deviation. ANOVA to compare data; different letters indicate statistical differences between wines of all treatments at the same time (Tukey's test, <0.05).

Table 2. Concentration of volatile compound in control and HHP treated red wines Cabernet Sauvignon

	Control	200 MPa			400 MPa			600 MPa		
		5 min	15 min	25 min	5 min	15 min	25 min	5 min	15 min	25 min
Esters (mg/L)										
ethyl acetate	37,34±1,82^{b}	37,28±0,65^{b}	36,51±0,78^{ab}	35,79±1,68^{ab}	35,39±0,90^{ab}	34,29±2,27^{ab}	34,10±0,95^{ab}	33,43±0,80^{ab}	33,73±1,87^{ab}	32,45±1,90^{a}
ethyl butyrate	0,44±0,03^{a}	0,41±0,04^{a}	0,38±0,03^{a}	0,39±0,01^{a}	0,38±0,06^{a}	0,38±0,06^{a}	0,38±0,01^{a}	0,37±0,02^{a}	0,36±0,02^{a}	0,34±0,05^{a}
ethyl hexanoate	0,42±0,01^{c}	0,39±0,01^{c}	0,36±0,03^{bc}	0,31±0,04^{ab}	0,30±0,02^{ab}	0,28±0,02^{a}	0,27±0,01^{a}	0,27±0,04^{a}	0,26±0,01^{a}	0,26±0,01^{a}
ethyl octanoate	0,19±0,03^{d}	0,15±0,02^{cd}	0,12±0,03^{bc}	0,13±0,02^{abc}	0,11±0,02^{abc}	0,09±0,01^{ab}	0,08±0,01^{a}	0,09±0,00^{ab}	0,09±0,00^{ab}	0,08±0,01^{ab}
ethyl decanoate	0,09±0,02^{b}	0,07±0,05^{ab}	0,05±0,02^{ab}	0,04±0,02^{ab}	0,03±0,01^{a}	0,02±0,01^{a}	0,02±0,01^{a}	0,02±0,01^{a}	0,01±0,01^{a}	0,01±0,01^{a}
diethyl succinate	0,61±0,03^{a}	0,59±0,02^{a}	0,60±0,04^{a}	0,57±0,13^{a}	0,56±0,02^{a}	0,54±0,03^{a}	0,53±0,05^{a}	0,53±0,05^{a}	0,51±0,09^{a}	0,51±0,02^{a}
i-butyl acetate	0,07±0,01^{c}	0,06±0,00^{ba}	0,06±0,00^{bc}	0,06±0,00^{bc}	0,06±0,00^{bc}	0,06±0,00^{ac}	0,05±0,00^{ab}	0,04±0,00^{a}	0,06±0,00^{bc}	0,05±0,00^{ab}
i-amyl acetate	0,63±0,05^{d}	0,61±0,05^{d}	0,60±0,05^{d}	0,57±0,05^{d}	0,56±0,04^{cd}	0,54±0,04^{bcd}	0,47±0,01^{abc}	0,45±0,02^{a}	0,46±0,02^{ab}	0,44±0,02^{a}
hexyl acetate	0,02±0,01^{b}	0,01±0,00^{ab}	0,01±0,01^{ab}	0,00±0,01^{ab}	0,00±0,01^{ab}	0,00±0,01^{ab}	0,00±0,00^{a}	0,01±0,00^{ab}	0,01±0,00^{ab}	0,01±0,01^{ab}
2-phenylethyl acetate	0,04±0,01^{a}	0,04±0,01^{a}	0,04±0,00^{a}	0,05±0,02^{a}	0,04±0,00^{a}	0,04±0,01^{a}	0,04±0,00^{a}	0,04±0,01^{a}	0,03±0,01^{ab}	0,03±0,01^{b}
Higher alcohols (mg/L)										
amyl alcohol	150,24±4,89^{a}	150,12±5,55^{a}	150,61±6,91	153,97±0,31^{a}	158,53±1,17^{a}	160,38±4,83^{a}	159,01±4,25^{a}	160,53±4,48^{a}	159,19±4,02^{a}	161,87±5,09^{a}
2-phenylethanol	27,35±1,70^{bc}	27,48±0,69^{c}	26,45±1,75	25,18±0,03^{abc}	24,01±2,30^{abc}	23,37±1,79^{abc}	23,85±2,98^{abc}	22,19±0,20^{a}	22,77±0,41^{ab}	22,02±2,26^{a}
1-hexanol	1,11±0,07^{a}	1,13±0,02^{ab}	1,21±0,02	1,21±0,02^{ab}	1,20±0,04^{ab}	1,23±0,04^{b}	1,21±0,06^{ab}	1,20±0,02^{ab}	1,21±0,05^{ab}	1,24±0,04^{b}
Fatty acids (mg/L)										
hexanoic acid	0,87±0,05^{a}	0,87±0,01^{a}	0,85±0,13	0,84±0,03^{a}	0,82±0,08^{a}	0,84±0,02^{a}	0,83±0,05^{a}	0,80±0,13^{a}	0,75±0,02^{a}	0,73±0,02^{a}
octanoic acid	2,62±0,11^{a}	2,62±0,23^{a}	2,59±0,08	2,54±0,06^{a}	2,56±0,12^{a}	2,55±0,11^{a}	2,54±0,08^{a}	2,57±0,28^{a}	2,54±0,06^{a}	2,49±0,22^{a}
decanoic acid	0,39±0,04^{e}	0,36±0,02^{de}	0,34±0,02	0,32±0,06^{bcde}	0,29±0,03^{abcd}	0,28±0,02^{abc}	0,26±0,02^{ab}	0,26±0,01^{ab}	0,25±0,02^{a}	0,26±0,02^{ab}
Terpenes (μg/L)										
linalool	7,67±0,02^{a}	7,65±0,16^{a}	7,67±0,10	7,69±0,08^{a}	7,69±0,33^{a}	7,67±0,19^{a}	7,53±0,21^{a}	7,48±0,04^{a}	7,45±0,06^{a}	7,41±0,05^{a}
Aldehydes (μg/L)										
benzaldehyde	259,98±1,94^{a}	256,50±5,38^{a}	254,99±6,63	253,75±3,47^{a}	253,07±0,79^{a}	253,54±3,15^{a}	252,37±1,95^{a}	252,72±6,26^{a}	251,12±5,76^{a}	251,67±3,70^{a}

Data presented as average value of three analytical repetitions (N=3) ± standard deviation. ANOVA to compare data; different letters indicate statistical differences between wines of all treatments at the same time (Tukey's test, <0.05).

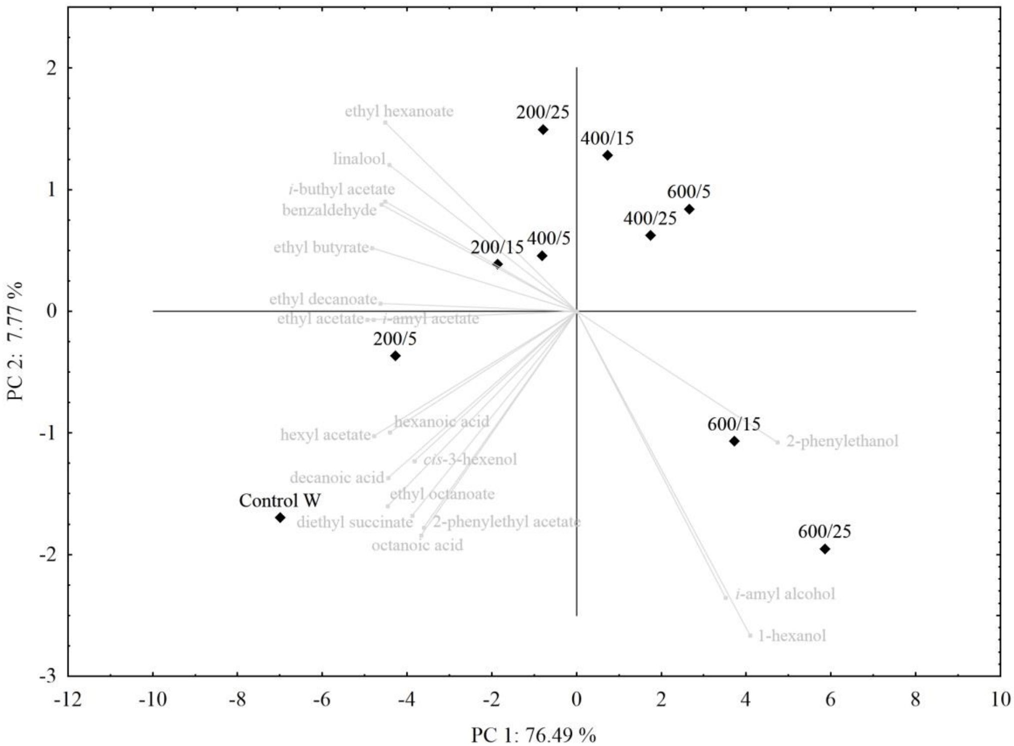


Fig. 1. Distribution of the white wine Graševina in two dimensional coordinate system defined by first two principal components (PC1 and PC2) according to applied HHP treatments

Projection of red wine Cabernet Sauvignon samples as well as analyzed aroma variables in the two-dimensional coordinate system defined by first two variables, explaining 86.3 %, is shown in Figure 2. First variable, that explain 73.37 % of the total variance (PC 1), showed strong negative correlations with the content of the ethyl acetate (-0.97), ethyl butyrate (-0.94), *i*-amyl acetate (-0.89), ethyl hexanoate (-0.98), *cis*-3-hexenol (-0.76), ethyl octanoate (-0.94), benzaldehyde (-0.92), linalool (-0.77), ethyl decanoate (-0.96), diethyl succinate (-0.89), hexanoic acid (-0.86), octanoic acid (-0.88), decanoic acid (-0.96) and 2-phenylethanol (-0.97). Moreover, PC 1 highly positively correlated with volatile amyl acohol (0.93) and 1-hexanol (0.85). PC 2 showed a negative correlation with 2-phenyelthyl acetate (-0.83) and linalool (-0.55) as well as positive correlation with hexyl acetate (0.87).

Separation of control and HHP treated red wine samples according to PCA analysis are presented in Figure 2. As it can be seen, control wine sample (Control R) of Cabernet Sauvignon and the wine samples pressurized by 200 MPa during 5, 15 and 25 minutes were placed on the left side of the first factorial plane and displaced from all treated wines due to higher concentrations of compounds which correlate negatively with first factorial plane. Red wine samples pressurized by 400 and 600

MPa were placed in fourth and first quadrant since they are characterized by higher concentration of volatile compound which correlate positively with the PC 1, primarily higher alcohols.

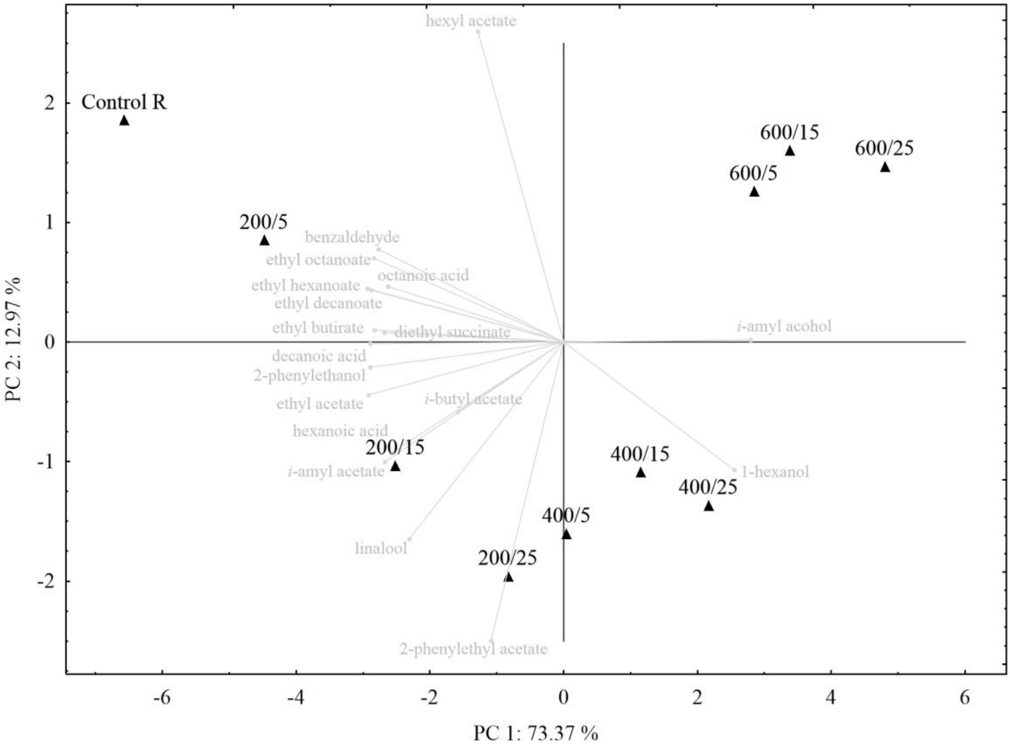


Fig. 2. Distribution of the red wine Cabernet Sauvignon in two dimensional coordinate system defined by first two principal components (PC1 and PC2) according to applied HHP treatments

CONCLUSION

High hydrostatic treatment influenced a slight change in concentrations of aroma compounds, primarily decrease of esters, volatile fatty acids and terpenes, while slight increase in concentration of higher alcohols was observed. Hence, this technique potentially could be very important in wine technology, especially in terms of wine production with lower sulfur dioxide additions. But, it is necessary to investigate long-term effect of this technique on overall quality of wine, including aroma and polyphenolic compounds, as well as sensory characteristics.

ACKNOWLEDGMENTS

This work was supported by grants from the Croatian Science Foundation project „New enological tools for the reduction of sulfur dioxide and production of high-quality wine“ (IP-09-2014-3796).

REFERENCES

Briones-Labarca, V., Perez-Wom, M., Habib, G., Giovagnoli-Vicuña, C., Cañas-Sarazua, R., Tabilo-Munizaga, G., Salazar, F. N. (2017): Oenological and Quality Characteristic on Young White Wines (Sauvignon Blanc): Effects of High Hydrostatic Pressure Processing. J. Food Qual. 2017, 1-12.

Buzrul S. (2012): High hydrostatic pressure treatment of beer and wine. Innovative Food Science and Emerging Technologies, 13, 1-12.

Corrales, M., Butz, P., Tauscher, B. (2008): Anthocyanin condensation reactions under high hydrostatic pressure. Food Chemistry, 110(3), 627-635.

Lambert, Y., Demazeau, G., Largeteau, A., & Bouvier, J. M. (1999): Changes in aromatic volatile composition of strawberry after high pressure treatment. Food Chemistry, 67(1), 7-16.

Mok, C., Song, K. T., Park, Y. S., Lim, S., Ruan, R., Chen, P. (2006): High Hydrostatic Pressure Pasteurization of Red Wine. Journal of Food Science, 71, 265-269.

Morata, A., Benito, S., González, M. C., Palomero, F., Tesfaye, W., Suárez-Lepe, J. A. (2012): Cold pasteurisation of red wines with high hydrostatic pressure to control Dekkera/Brettanomyces: effect on both aromatic and chromatic quality of wine. European Food Research and Technology, 235, 147-154.

Morata, A., Loira, I., Vejarano, R., Bañuelos, M. A, Sanz, P. D., Otero, L., Suárez-Lepe, J. A. (2015): Grape Processing by High Hydrostatic Pressure: Effect on Microbial Populations, Phenol Extraction and Wine Quality. Food and Bioprocess Technology, 8, 277-286.

Norton, T., Sun, D. W. (2008): Recent advances in the use of high pressure as an effective processing technique in the food industry. Food and Bioprocess Technology, 1(1), 2-34.

Puig, A., Vilavella, M., Daoudi, L., Guamis, B., Minguez, S. (2003): Microbiological and biochemical stabilization of wines using the high pressure technique. Bulletin de l′O.I.V., 76, 596-617.

Santos, M. C., Nunes, C., Jourdes, M., Teissedre, P. L., Rodrigues, A., Amado, O., Saraiva, J. A., Coimbra, M. A. (2016): Evaluation of the potential of high pressure technology as an enological practice for red wines. Innovative Food Science and Emerging Technologies, 33, 76-83.

Santos, M. C., Nunes, C., Rocha, M. A. M., Rodrigues, A., Rocha, S. M., Saraiva, J. A., Coimbra, M. A. (2013): Impact of high pressure treatments on the physicochemical properties of a sulphur dioxide-free white wine during bottle storage: Evidence for Maillard reaction acceleration. Innovative Food Science and Emerging Technologies, 20, 51-58.

Santos, M. C., Nunes, C., Rocha, M. A. M., Rodrigues, A., Rocha, S. M., Saraiva, J. A., Coimbra, M. A. (2015): High pressure treatments accelerate changes in volatile composition of sulphur dioxide-free wine during bottle storage. Food Chemistry, 188, 406-414.

Sun, X., Li, L., Ma, T., Zhao, F., Yu, D., Huang, W., Zhan, J. (2016): High hydrostatic pressure treatment: An artificial accelerating aging method which did not change the region and variety non-colored phenolic characteristic of red wine. Innovative Food Science and Emerging Technologies, 33, 123-134.

Tao, Y., Sun, D. W., Górecki, A., Błaszczak, W., Lamparski, G., Amarowicz, R., Fornal, J., Jeliński, T. (2012): Effects of high hydrostatic pressure processing on the physicochemical and sensorial properties of a red wine. Innovative Food Science and Emerging Technologies, 16, 409-416.

Tomašević, M., Gracin, L., Ćurko, N., Ganić, K. K. (2017): Impact of pre-fermentative maceration and yeast strain along with glutathione and SO 2 additions on the aroma of *Vitis vinifera* L. Pošip wine and its evaluation during bottle aging. LWT-Food Science and Technology, 81, 67-76.

INFLUENCE OF HIGH VOLTAGE ELECTRICAL DISCHARGE PLASMA TREATMENT ON THE PHYSICOCHEMICAL CHARACTERISTICS OF WINE*

UTJECAJ VISOKONAPONSKOG ELEKTRIČNOG PRAŽNJENJA-HLADNE PLAZME NA FIZIKALNO-KEMIJSKE KARAKTERISITKE VINA

Katarina Lukić[1], Marina Tomašević[1], Tomislava Vukušić[1], Karla Kelšin[1], Leo Gracin[1], Karin Kovačević Ganić[1]

Original scientific paper

Rezime

Cilj ovoga istraživanja bio je ispitati utjecaj visokonaponskog električnog pražnjenja-hladne plazme na fizikalno-kemijske karakteristike vina, zbog potencijalne primjene ove tehnologije u proizvodnji vina. Istražen je utjecaj procesnih parametara hladne plazme, frekvencije (60, 90, 120 Hz) i trajanja tretmana (3, 5, 10 min) pri pozitivnom polaritetu na koncentraciju otopljenog kisika, koncentracije slobodnog i ukupnog sumporovog dioksida (SO_2), te električnu provodljivost u bijelom i crnom vinu. Neposredno nakon tretmana provedene su analize, gdje je koncentracija otopljenog kisika određena pomoću uređaja za mjerenje kisika, koncentracija slobodnog i ukupnog SO_2 potenciometrijskom titracijom, dok je konduktometrom izmjerena električna provodljivost. Dobiveni rezultati pokazali su da primjenom tretmana hladnom plazmom dolazi do smanjenja koncentracije otopljenog kisika i ukupnog SO_2 u usporedbi sa kontrolnim vinima. S druge strane, električna provodljivost se povećala nakon primijenjenog tretmana, dok se koncentracija slobodnog SO_2 ili smanjila ili povećala. Također, rezultati su pokazali da frekvencija i trajanje tretmana značajno utječu na fizikalno-kemijske karakteristike vina.

Ključne riječi: *hladna plazma, vino, fizikalno-kemijski parametri*

Summary

The aim of proposed research was to study the influence of high voltage electrical plasma discharges on the physicochemical characteristics of wines, due to the potential use of this technique in winemaking. The effects of plasma discharge frequency (60, 90, 120 Hz) and treatment duration (3, 5, 10 min) with positive electrode polarity on the changes in concentrations of dissolved oxygen, free and total

---

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina

[1] Prehrambeno-biotehnološki fakultet, Sveučilište u Zagrebu/ Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia

sulfur dioxide (SO_2) and electrical conductivity in white and red wines were investigated. The analyses were done immediately after treatment, where the dissolved oxygen was measured by oxygen-meter, free and total SO_2 by potentiometric titration while conductometer was used for electrical conductivity measurements. The results showed that applied treatments influenced the decrease in concentration of dissolved oxygen and total SO_2 in comparison to control wines. On the other hand, electrical conductivity increased after applied treatment, while concentration of free SO_2 was either decreased or increased. The results also showed that physicochemical characteristics of wines were significantly affected by frequency as well as processing time.

Key words: *high voltage electrical discharge plasma, wine, physicochemical characteristics*

INTRODUCTION

The new innovative technologies, such as high hydrostatic pressure, pulsed electric fields, high voltage arc discharge and non-thermal plasma, are today of great interest in food industry. Among these novel technologies, the application of plasma technology to wine has not been investigated so far. Most of the studies have been carried out on inactivation effect of various plasma treatments on microorganisms (Ziuzina *et al.*, 2013; Shi *et al.*, 2011; Vukušić *et al.*, 2016; Misra and Jo, 2017). Recently, the focus has begun to shift towards impact of plasma on food constituents (Grzegorzewski *et al.*, 2011; Misra *et al.*, 2015; Bursać Kovačević *et al.*, 2016; Elez Garofulić *et al.*, 2015; Ramazzina *et al.*, 2016), which is still insufficiently explained. Because of the lack of knowledge on the primary modes of action and on the effects on sensory and nutritional properties of the products, the use of plasma technology for food processing has not been yet allowed (Niemira, 2012). The plasma is a non-thermal technology, which is described as partially or completely ionized gas with electrical, chemical and physical properties (Petitpas *et al.*, 2007). The plasma can be produced by many methods such as electric discharges (corona, spark, glow, arc, microwave discharge, plasma jets and radio frequency plasma) and shocks (electrically, magnetically and chemically driven) (Petitpas *et al.*, 2007). The primary effects of electrical discharges are the UV radiation and the generation of reactive chemical species by the plasma ionization process (Niemira, 2012).The inactivation efficiency of plasma is associated with large number of variables, primarily with employed plasma sources and process parameters as well as with the characteristics of treated product (Misra and Jo, 2017). Apart from the nutritional and sensory quality, the physicochemical parameters are often employed for judging the quality of products. Basic physicochemical parameters such as pH, sulfur dioxide (SO_2) and others are generally used to define and to express wine quality (García Martín and Sun, 2013). In wine, oxygen can influence the composition and quality of wine drastically due to its involvement in various reactions (Du Toit *et al.*, 2006).

The measurements of dissolved oxygen in wine are significant because the contact between wine and oxygen is a critical point during the wine production (Castellari *et al*., 2004). Another important physicochemical parameter of wine is the electrical conductivity because of its good correlation with pH and assimilable nitrogen during fermentation (Colombié *et al*., 2008). Electrical conductivity is defined as the ability of a solution to conduct electric current (Colombié *et al*., 2008). Thus, the aim of this research was to study the influence of the plasma treatment, as possible alternative technique to reduce the addition of SO_2 in wine, on the changes of previously mentioned physicochemical parameters (dissolved oxygen, electrical conductivity and total/free SO_2 concentrations).

MATERIAL AND METHODS

Material

The white wine Graševina (*Vitis Vinifera L.)* and red wine Cabernet Sauvignon (*Vitis Vinifera L.)* used in this study were acquired from the winery Erdutski vinogradi d.o.o., harvest 2016, in Erdut, Croatia. Physicochemical properties of Graševina were: 11.4 vol %, total acidity (as tartaric acid) 5.1 g/L, volatile acidity (as acetic acid) 0.31 g/L, reducing sugars 2.8 g/L, pH 3.37, malic acid 1.2 g/L, lactic acid 0.3 g/L, while those of Cabernet Sauvignon were: 13.1 vol %, total acidity (as tartaric acid) 5.3 g/L, volatile acidity 0.61 g/L, reducing sugars 4.1 g/L, pH 3.46, malic acid 0.1 g/L, lactic acid 1.3 g/L.

Chemicals

Sulfuric acid 1/3 (941), sodium hydroxide 2N (908), sulfuric acid 1/10 (932) and iodide/iodate N/64 (921) were purchased from LDS Laboratoires Dujardin-Salleron (Noizay, France).

Methods

The plasma treatments of wine

The plasma treatments were conducted in a 1000 mL glass vessel with a point to point electrode configuration in a so-called hybrid reactor with discharges in and above the liquid. The plasma was generated by high-voltage (HV) pulsed power supply (Spellman, UK) by charging a load capacitor of 1.13 nF to up to 30 kV and then discharging the stored charge into the plasma reactor via a rotating spark gap. The voltage in the plasma reactor was measured and recorded using a Tektronix P6015A high voltage probe connected to a Hantek DS05202BM oscilloscope (data not shown). The experiments were performed at positive polarity and argon (purity 99.99%; Messer Croatia, Zagreb, Croatia) was bubbled trough stainless steel needle (Micrlolance TM 3.81 cm) at the gas flow of 4 L/min. 300 mL of wine was treated with plasma running at the combination of following processing parameters: frequency at 60, 90 and 120 Hz and treatment duration of 3, 5 and 10 min. The temperature and pH value of samples before and after the plasma treatment were monitored (data not shown). Before treatment, all samples were at the room temperatures of 21 ± 1 °C, while after the plasma exposure temperature raised up to 6 °C, depending on the treatment duration and applied frequency. The pH value of wines maintained relatively constant ranging from 3.1 to 3.4. After treatments, wine samples were subjected to physicochemical analysis.

Physicochemical analysis

In control and treated wines, immediately after plasma treatments, the concentrations of dissolved oxygen, free and total SO_2 and electrical conductivity were measured. The measurement of dissolved O_2 concentration was carried out using the oxygen measurement device (Nomasense O2 P6000, Nomacorc, Belgium), which is based on luminance principle. The device corrects the concentration of oxygen in terms of sugar and alcohol content and the temperature of the wine. Determination of dissolved oxygen was carried out immediately after treatment with plasma using an immersion probe with a detection limit of 15 μg/L of oxygen. The measurement of the free and total SO_2 concentration was performed on a SO_2 measurement device (LDS Sulfilyser, Laboratoires Dujardin-Salleron, Noizay, France) by titration with iodide/iodate solution whereby the iodine was reduced and SO_2 oxidized, with the potentiometric determination of the titration point via the LED indicator. Electrical conductivity was measured using a digital pH-meter HANNA edge (HANNA instruments, Croatia, Zagreb, Croatia). The measurement was performed by immersing the electrode for measuring electrical conductivity in the sample and after the stabilization the measured values were recorded.

Statistical analysis

Statistical analysis was carried out using analysis of variance (ANOVA) of Statistica V.10 software (Statsoft Inc., Tulsa, USA). Tukey's HSD test was used as comparison test when samples were significantly different after ANOVA ($p<0.05$).

RESULTS AND DISCUSSION

The results of the effects of various plasma treatments on the physicochemical parameters of wine are presented in Tables 1 (Graševina) and 2 (Cabernet Sauvignon). The results showed that physicochemical characteristics of wines were significantly influenced ($p \leq 0.05$) by the plasma treatment. As can be seen in Table 1, after different plasma treatments, the concentration of dissolved oxygen and total SO_2 values in white wine were reduced, while the concentration of free SO_2 was highly variable and independent of applied processing parameters. On the other hand, the value of electrical conductivity of the treated wine increased after the applied treatment compared to the untreated (control) wine. Regarding the SO_2 in wine, it is important to control its concentration after applied plasma treatments because SO_2 has antioxidant and antimicrobial effects on wine (Usseglio – Tomasset, 1992; Oliveira *et al.*, 2011; Ugliano, 2013; Guerrero and Cantos – Villar, 2015).

Except the SO_2, the concentration of dissolved oxygen also represents a crtical parameter for the control of various processing treatments (Castellari *et al.*, 2004). Among these, the reaction of SO_2 with oxygen is slow and has a crucial role in SO_2 antioxidant activity (Ugliano, 2013). It is known that the amount of SO_2 that binds with other substances in wine, or that remains free, depends on the wine temperature and pH. These observations can be related to changes in temperature of samples after the plasma exposure and the fact that the plasma is oxidative method (Vukušić, 2016). From the processing parameters, the treatment duration had a greater impact on physicochemical parameters of wines, but also the influence of plasma discharge frequency should not be neglected. These data show that the largest decrease in the examined parameters (dissolved oxygen and total SO_2 concentrations) occurred at treatment at 120 Hz for 10 minutes. Furthermore, the highest reduction of free SO_2 concentration was observed at treatment at 90 Hz for 10 minutes, while the highest concentration of free SO_2 was determined in the sample treated at a frequency of 60 Hz for 3 minutes. It has been demonstrated that by increasing the plasma frequency a large number of discharges occur leading to the generation of numerous radicals. In addition, by creating a strong photoionization effect the part of energy transfers to the surrounding medium and warms it (Vukušić, 2016). Regarding the electrical conductivity of the treated wine, the values increased along with increasing the treatment duration, but also by increasing the plasma discharge frequency. By increasing the electrical conductivity, the voltage required for the initiation of the discharge reduces (Zhu *et al.*, 2009). Also, the electron density in the liquid increases. That is why the electricity discharge is larger, resulting in plasma higher density and temperature and more intense UV radiation (Locke *et al.*, 2006). When the conductivity values are above 5 mS/cm plasma bullets are shorter than 1 mm and strong acoustic waves are created (Šunka, 2001). Although the physical processes are more intense, shorter bullets also means that the smaller volume of the liquid will be in contact with the plasma (Vukušić, 2016). Furthermore, the similar effect of plasma treatments was also observed in red wine (Table 2), where the highest reduction of dissolved oxygen and total SO_2 concentrations, but also the highest concentration of free SO_2 occurred in the sample treated at 120 Hz for 10 minutes. Moreover, the highest reduction of free SO_2 concentration was observed at treatment at 90 Hz for 10 minutes. The values of electrical conductivity determined in red wine also increased by increasing plasma processing parameters. Overall, the characteristics of the obtained wines showed that the applied plasma treatments resulted in wines with different physicochemical parameters compared to the untreated wines.

Table 1. Effects of plasma treatments on physicochemical parameters in white wine Graševina

Treatments	Dissolved oxygen (mg/L)	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Conductivity (µS/cm)
Untreated (control)	3.03±0.04^{f}	12.67±0.58^{bc}	65.00±0.00^{d}	1409.33±6.03^{a}
60 Hz/3 min	2.80±0.02^{e}	14.67±0.58^{d}	65.00±0.00^{d}	1528.00±15.10^{b}
90 Hz/3 min	2.53±0.09^{c}	12.67±0.58^{d}	60.83±1.44^{b}	1570.67±3.06^{c}

120 Hz/3 min	2.17±0.04^{c}	12.67±0.58^{bc}	62.50±0.00^{bcd}	1614.00±3.61^{d}
60 Hz/5 min	2.96±0.06^{f}	12.67±0.58^{bc}	64.17±1.44^{cd}	1576.33±12.50^{c}
90 Hz/5 min	2.56±0.09^{d}	13.67±0.58^{cd}	60.00±0.00^{ab}	1556.33±13.32^{c}
120 Hz/5 min	2.14±0.03^{bc}	11.33±0.58^{ab}	60.83±1.44^{b}	1627.00±8.89^{d}
60 Hz/10 min	2.50±0.02^{d}	11.67±0.58^{ab}	61.67±1.44^{bc}	1583.00±7.94^{c}
90 Hz/10 min	2.01±0.02^{ab}	10.33±0.58^{a}	60.00±0.00^{ab}	1628.00±5.00^{d}
120 Hz/10 min	1.97±0.06^{a}	11.33±0.58^{ab}	57.50±0.00^{a}	1664.67±9.81^{e}

Data presented as average value of three analytical repetitions with stadard deviation. ANOVA to compare data; different letters indicate statistical differences between wines of all treatments at the same time (Tukey's test, $p < 0.05$).

Table 2. Effects of plasma treatments on physicochemical parameters in red wine Cabernet Sauvignon

Treatments	Dissolved oxygen (mg/L)	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Conductivity (µS/cm)
Untreated (control)	1.64±0.01^{e}	16.67±0.58^{c}	40.00±0.00^{d}	1756.00±1.00^{a}
60 Hz/3 min	1.55±0.04^{d}	13.67±0.58^{ab}	39.67±0.58^{d}	1829.67±1.53^{de}
90 Hz/3 min	1.49±0.02^{cd}	14.67±0.58^{b}	42.00±0.87^{e}	1833.67±1.53^{e}
120 Hz/3 min	1.36±0.03^{ab}	13.67±0.58^{ab}	37.00±0.87^{c}	1820.33±1.53^{c}
60 Hz/5 min	1.40±0.02^{ab}	17.67±0.58^{cd}	30.33±0.58^{b}	1806.33±4.04^{b}
90 Hz/5 min	1.51±0.02^{d}	13.67±0.58^{ab}	30.00±0.00^{b}	1830.33±1.53^{de}
120 Hz/5 min	1.54±0.04^{d}	14.67±0.58^{b}	30.00±0.00^{b}	1811.33±3.21^{b}
60 Hz/10 min	1.33±0.03^{a}	18.33±0.58^{cd}	27.67±0.29^{a}	1823.00±3.61^{c}
90 Hz/10 min	1.41±0.02^{bc}	12.67±0.58^{a}	29.67±0.58^{b}	1830.33±2.52^{de}
120 Hz/10 min	1.33±0.03^{a}	18.67±0.58^{d}	27.00±0.87^{a}	1863.67±3.51^{f}

Data presented as average value of three analytical repetitions with stadard deviation. ANOVA to compare data; different letters indicate statistical differences between wines of all treatments at the same time (Tukey's test, $p < 0.05$).

CONCLUSION

In summary, this study showed that plasma treatments have influenced the physicochemical characteristics of wines. Compared to untreated wines, plasma treatments resulted in changes of all measured parameters, namely in reduction of dissolved oxygen and total SO_2. The concentration of free SO_2 was either decreased or increased, while electrical conductivity of wines increased after applied plasma treatments. Altogether, our results demonstrated the importance of determining the changes in wine physicochemical parameters after exposure to plasma treatments. Finally, these data are crucial precondition for possible application of plasma technology in wine industry. However, these parameters only provide overall quality of the wine. Future studies on the current topic are therefore required, particularly on sensory and chemical characteristics (phenolic and aroma compounds), in order to evaluate the plasma efficiency in winemaking.

ACKNOWLEDGMENTS

This work was supported by grants from the Croatian Science Foundation project „New enological tools for the reduction of sulfur dioxide and production of high-quality wine" (IP-09-2014-3796).

REFERENCES

Bursać Kovačević, D., Putnik, P., Dragović-Uzelac, V., Pedisić, S., Režek Jambrak, A., Herceg, Z. (2016): Effects of Cold Atmospheric Gas Phase Plasma on Anthocyanins and Color in Pomegranate Juice. Food Chemistry, 190, 317-323.

Castellari, M., Simonato, B., Tornielli, G. B., Spinelli, P., Ferrarini, R. (2004): Effects of different enological treatments on dissolved oxygen in wines. Italian Journal of Food Science, 16(3), 387-397.

Colombié, S., Latrille, E., Sablayrolles, J. M. (2008): Interest of on-line monitoring electrical conductivity during wine fermentation. European Food Research and Technology, 226(6), 1553-1557.

Du Toit, W. J., Marais, J., Pretorius, I. S., Du Toit, M. (2006): Oxgyen in must and wine: a review. South African Journal of Enology and Viticulture, 27(1), 76-94.

Elez Garofulić, I., Režek Jambrak, A., Milošević, S., Dragović-Uzelac, V., Zorić, Z., Herceg, Z. (2015): The effect of gas phase plasma treatment on the anthocyanin and phenolic acid content of sour cherry Marasca (*Prunus cerasus* var. *Marasca*) juice. LWT – Food Science and Technology, 62(1), 894-900.

Grzegorzewski, F., Ehlbeck, J., Schlüter, O., Kroh, L. W., Rohn, S. (2011): Treating lamb's lettuce with a cold plasma e influence of atmospheric pressure Ar plasma immanent species on the phenolic profile of Valerianella locusta. Food Science and Technology, 44 (10), 2285-2289.

Guerrero, R. F., Cantos-Villar, E. (2015): Demonstrating the efficiency of sulphur dioxide replacements in wine: a parameter review. Trends in Food Science & Technology, 42(1), 27-43.

Locke, B. R., Sato, M., Sunka, P., Hoffmann, M. R., Chang, J.-S. (2006): Electrohydraulic Discharge and Nonthermal Plasma for Water Treatment. Industrial & Engineering Chemistry Research, 45, 882-905.

Martín, J. F. G., Sun, D. W. (2013): Ultrasound and electric fields as novel techniques for assisting the wine ageing process: The state-of-the-art research. Trends in food science & technology, 33(1), 40-53.

Misra, N. N., Jo, C. (2017): Applications of cold plasma technology for microbiological safety in meat industry. Trends in Food Science & Technology, 64, 74-86.

Misra, N. N., Pankaj, S. K., Frias, J. M., Keener, K. M., Cullen, P. J. (2015): The effects of nonthermal plasma on chemical quality of strawberries. Postharvest biology and technology, 110, 197-202.

Niemira, B. A. (2012): Cold plasma decontamination of foods. Annual review of food science and technology, 3, 125-142.

Oliveira, C. M., Ferreira, A. C. S., De Freitas, V., Silva, A. M. S. (2011): Oxidation mechanisms occurring in wines. Food Research International, 44(5), 1115-1126.

Petitpas, G., Rollier, J.-D., Darmon, A., Gonzalez-Aguilar, J. Metkemeijer, R., Fulcheri, L. (2007): A comparative study of non-thermal plasma assisted reforming technologies. International Journal of Hydrogen Energy, 32, 2848-2867.

Ramazzina, I., Tappi, S., Rocculi, P., Sacchetti, G., Berardinelli, A., Marseglia, A., Rizzi, F. (2016): Effect of Cold Plasma Treatment on the Functional Properties of Fresh-Cut Apples. Journal of agricultural and food chemistry, 64(42), 8010-8018.

Shi, X. M., Zhang, G. J., Wu, X. L., Li, Y. X., Ma, Y., Shao, X. J. (2011): Effect of low-temperature plasma on microorganism inactivation and quality of freshly squeezed orange juice. IEEE Transcations on Plasma Science, 39(7), 1591–1597.

Šunka, P. (2001): Pulse electrical discharges in water and their applications. Physics of plasmas, 8(5), 2587-2594.

Ugliano, M. (2013): Oxygen contribution to wine aroma evolution during bottle aging. Journal of agricultural and food chemistry, 61(26), 6125-6136.

Usseglio-Tomasset, L. (1992): Properties and use of sulphur dioxide. Food Additives and Contaminants, 9(5), 399-404.

Vukušić, T. (2016): Application of cold plasma in processing of fruit juices. (Unpublished PhD thesis), Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb.

Vukušić, T., Shi, M., Herceg, Z., Rogers, S., Esifaee, P., Thagard, S. M. (2016): Liquid-phase electrical discharge plasmas with a silver electrode for inactivation of a pure culture of Escherichia coli in water. Innovative Food Science and Emerging Technologies, doi: http://dx.doi.org/10.1016/j.ifset.2016.07.007.

Zhu, T., Zhang, Q., Jia, Z., Yang, L. (2009): The effect of conductivity on streamer initiation and propagation between dielectric-coated sphere-plate electrodes in water. IEEE Transactions on Dielectrics and Electrical Insulation, 16(6), 1552-1557.

Ziuzina, D., Patil, S., Cullen, P. J., Keener, K. M., Bourke, P. (2013): Atmospheric cold plasma inactivation of Escherichia coli in liquid media inside a sealed package. Journal of Applied Microbiology, 114(3), 778–787.

INFLUENCE OF TOBACCO BLEND COMPOSITION ON POLYCYCLIC AROMATIC HYDROCARBONS FORMATION IN CIGARETTE SMOKE*

UTICAJ SASTAVA DUHANSKE MJEŠAVINE NA FORMIRANJE POLICIKLIČNIH AROMATSKIH UGLJIKOVODIKA U DIMU CIGARETE

Nermina Djulančić[1], Vesna Radojičić[2], Marija Srbinoska[3], Jasmina Tahmaz[1]

Original scientific paper

Rezime

Odabir duhana prvi je korak u proizvodnji cigareta koje u dim emituju smanjenu količinu policikličnih aromatskih ugljhikovodika (PAH). Cilj ovog rada bio je ispitati uticaj tri osnovna tipa duhana: duhan sušen ambijentalnim vazduhom (AC-Berlej), duhan sušen u kontrolisanim uslovima (FC-Virdžinija), duhan sušen na suncu (SC-orijentalni duhan), rekonstituisani duhan (RT) i dodatak ekspandovanog nerva na formiranje PAH-ova u čestičnoj fazi glavne struje dima cigareta. Sve analize su urađene primjenom standardnih metoda. Dobijeni rezultati pokazali su da tip i količina duhana u mješavini imaju signifikantan uticaj na formiranje prosječnog sadržaja ukupnih i pojedinačnih PAH-ova u cigaretnom dimu. U dimu SC cigarete utvrđena je najviša količina (853,97 ng/cig) ukupnih PAH-ova. Najveću učinkovitost u smislu smanjenja sadržaja nikotina, tara i PAH-ova pokazao je dodatak ekspandovanog nerva u količini od 30%. Fizičke karakteristike cigarete i hemijski sastav dima, također, su imale vrlo snažan utjecaj na formiranje PAH-a.

Ključne riječi: *poliociklični aromatski ugljikovodici; FC duhan; AC duhan; SC duhan; folija; ekspandovani nerv; cigaretni dim*

Summary

Tobacco selection is the first step in the manufacturing of cigarettes with reduced emission of polycyclic aromatic hydrocarbons (PAH) contained in the smoke. The aim of this work was to examine the influence of three basic tobacco types: tobacco dried in ambient air (AC-Burley), tobacco dried in controlled conditions (FC-Virginia), tobacco dried in sun (SC-oriental tobacco), reconstituted tobacco (RT) and addition of expanded stems, on the formation of PAHs in particulate phase of the mainstream cigarette smoke. All analyzes have been done by using standardized methods. The

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina

[1] University of Sarajevo, Faculty of Agriculture and Food Science, Sarajevo, Bosnia and Herzegovina
[2] University of Belgrade, Faculty of Agriculture, Belgrade, Republic of Serbia
[3] University St. Kliment Ohridski – Bitola, Scientific Tobacco Institute –Prilep Former Yugoslav Republic of Macedonia

obtained results have shown that the type and the quantity of tobacco in the blends had significant effect on forming of the average content of total and individual PAHs in cigarette smoke. The greatest quantity (853,97 ng/cig.) of total PAHs was established in the smoke of SC cigarette. The highest efficiency in terms of reduction of the average content nicotine, tar and PAHs was demonstrated by the expanded stem in the quantity of 30%. Physical characteristics of the cigarette and chemical composition of smoke have also had a very strong impact on the PAHs production.

Key words: *polycyclic aromatic hydrocarbons; FC tobacco; AC tobacco; SC tobacco; recon; expanded stem; cigarette smoke*

INTRODUCTION

Tobacco smoke is a complex aerosol containing about 6000 components, which are classified into different groups of chemical compounds [1, 2]. A large number of compounds in tobacco smoke are considered to be harmful for the human health, whereas some are even carcinogenic. Polycyclic aromatic hydrocarbons (PAHs) are classified as very dangerous. The estimation has shown that there are 549 individual PAHs in the tobacco smoke [3], twelve of them being classified as carcinogens by IARC [4, 5]. The US Environmental Protection Agency (EPA) has also designated 16 PAHs as main environmental pollutants due to their toxicity and carcinogenic potential. PAHs are formed during incomplete combustion of a mixture of cigarettes [6]. Given the significant number of smokers in the world, tobacco smoke is considered to be a significant source of PAHs, consumed by smokers. Also, a large amount of these substances goes in the atmosphere [5], because the sidestream smoke of particulate phase contains 10-fold higher amounts of PAHs than the mainstream smoke [7].

Different cigarette brands using different ingredient blends, changes in cigarette design and manufacturing methods can influence the combustion efficiency of cigarettes and thus the mainstream and sidestream smoke delivery of different contaminants. Mainstream smoke is drawn by the smoker through the cigarette [8]. The particulate phase of mainstream cigarette smoke comprises between 4 and 9% of the total weight of cigarette smoke and is made up of many components including polycyclic aromatic hydrocarbons, tobacco specific nitrosamines, phenol and nicotine.

In order to protect the public health and the environment in general, it is crucially important to keep these contaminants at levels which are toxicologically acceptable, or, if possible, to reduce their amounts. Any technological process that contributes to PAHs reduction is a significant contribution to the protection of active and passive smokers. There are various methods for purification of tobacco smoke aimed at removing as much as possible the substances which are harmful for the health or could contribute to air pollutio. One method is the filtration of already formed smoke and the other one is the change of burning conditions in a way so as to obtain more complete burning and to prevent synthesis of harmful substances [9, 10, 11, 12]. Nevertheless, the tobacco selection is the first and the simplest step in the manufacturing of cigarettes with reduced emission of PAHs contained in the smoke. Since different types of tobacco produce different amounts of PAHs, the amount of PAHs in tobacco smoke can be controlled by the composition of mixtures.

The objectives of this research are: (1) Determination of the content of total and 8 individual PAHs in particulate matter of seven test-cigarettes contained either a single type of tobacco (Burley, Virginia, oriental, reconstituted) or a mixture of tobacco types and addition of expanded stems; (2) Identification of PAHs precursors in tobacco smoke; (3) Calculation of correlation coefficients between PAHs content and chemical composition of the analyzed blend; (4) Calculation of correlation coefficients between PAHs content and physical properties of cigarettes; (5) Calculation of correlation coefficients between PAHs content and chemical composition of tobacco smoke.

MATERIALS AND METHODS

The following tobacco and non-tobacco materials were used for the production of cigarettes analysed in this research: Oriental tobacco (SC) origin from Macedonia (grade unik I-III), crop 2012, Burley (AC), origin from Bosnia and Herzegovina (grade unik I-III), crop 2012; Virginia (FC), origin from Bosnia and Herzegovina (grade unik I-III), cropo 2012. Reconstituted tobacco (RT), RECON GC-2, manufactured by LEMAN, France and Expanded stem (50% FC, 50% AC).

The experimental cigarettes were made by industrial machines Hauni Protos 90E (Hauni Maschinenbau AG, Hamburg, Germany) according to the specifications presented in Table 1. To reduce complications associated with differing filter ventilation all examined cigarettes were unventilated. The following non-tobacco materials were used for the manufacturing of cigarettes analyzed in this research: Cigarette paper, permeability 42 CU (cm/min at 1 kPa), and tipping paper non-porous. Cellulose acetate filter 2.7 Y⁄35 000 and non-perforated cork paper TP 719 J. Cigarette side-seam glue TURMERLEIM DNA 12/5 G and tipping glue TURMERLEIM MAX I/S. Propylene glycol (Austria Tabak was used in the amount of 2.5 % of the total amount of tobacco (dry weight) for the purpose of improving the hygroscopic properties. The total cigarette length was 85 mm. The length of the filter was 20 mm. Cigarettes for testing the composition of the tobacco blend were sampled on a random basis by cutting along the cigarette rod and taking out the tobacco blend.

All seven tobacco cigarette samples (SC, AC, FC, RT, B_1, B_2 and B_3) were dried in an oven at a temperature of 40°C. After drying, the samples were milled, sieved through a 0.5 mm sieve and placed in amber glass jars. Nicotine and reducing sugars were determined by Continuous Flow Analysis using methods as described in the relevant ISO standards [13, 14], while total nitrogen was analyzed employing the Kjeldahl procedure [15]. Ash content was determined by using the standard AOAC method [16]. All analyses were performed in triplicate.

Determination of cigarette weight and draw resistance was performed on the Sodimat device (Sodim SAS, HAUNI, France). The module for draw resistance measuring was done according to the ISO 6565 method [17]. Twenty cigarettes from each sample were analyzed. All the analyses were conducted in triplicate. Cigarette hardness was set as a given parameter (67.95± 0.45) %. All test cigarettes had the same diameter (7.95± 0.012) mm.

Table 1. Sheme of experiment

Cigarette	Blend composition
SC	100% Oriental tobacco
AC	100% Burley
FC	100% Virginia
RT	100% RECON
B_1	American blend: 10% Oriental tobacco 30% Burley 60% Virginia
B_2	American blend: 5% Oriental tobacco 25% Burley 45% Virginia 10% RECON 15% Expanded stem
B_3	American blend: 5% Oriental tobacco 25% Burley 30% Virginia 10% RECON 30% Expanded stem

Cigarettes are conditioned for a minimum of 48 hours prior to use (60±2% relative humidity, 22±1°C) according to ISO 3402 [18]. Mainstream smoke total particulate matter was generated by following the ISO 3308 [19] smoking regime (60-s puff interval, 2-s puff duration, and 35-mL puff volume) and collected on Cambridge filter pads using a Borgwaldt RM 20/CSR smoking machine (Borgwaldt, Germany). The smoke condensate was extracted from Cambridge filter with methanol. The nicotine content of the total particulate matter is determined by gas chromatography as set out in ISO 10315 [20]. The water content of the total particulate matter shall be determined by gas chromatography as set out in ISO 10362-1 [21]. The NFDPM (or TAR) content is determined by subtracting the water content and the nicotine content from the total particulate matter as set out in ISO 4387 [22].

In this experiment, we measured the concentrations of the following individual PAHs: fluorene (Flu), pyrene (Pyr), benzo [*a*] anthracene (B[a]A), chrysene (Chry), benzo[*a*]pyrene (B[a]P), benzo[*b*]fluoranthene (B[b]F), benzo[*k*]fluoranthene (B[k]F) and dibenzo[a,h]anthracene (D[a,h]A) in mainstream smoke total particulate matter (TPM).

A total and individual PAHs was measured for each sample [23]. After smoking, methanol extraction was conducted on the total particulate matter. Extracts were dried using a Zymark Turbovap LV evaporator to 3 mL. A 200 μL aliquot was transferred to an autosampler vial and 1 μL was injected into a Gas Chromatograph 6890 Network GC System with mass detector 5973 Network Mass Selective Detector (MSD) (Hewlett-Packard Company, USA). For the extraction of PAHs pro-analysis-grade solvents methanol and individual PAHs used for standards were obtained from Sigma-Aldrich (USA).

Results were reported as mean ± standard deviation. (SD). The statistical analysis was performed by the SPSS 17 software. ANOVA with pot hoc test analysis based on

Tukey test was used to compare differences between samples [24]. The relationship the PAHs content in the smoke and the chemical components of tobacco and physical characteristics of cigarette were estimated by using the Pearson's correlation coefficient.

RESULTS AND DISCUSSION

Bearing in mind that the chemical composition of particulate phase (TPM) mainstream smoke cigarette (Table 3) depends on the chemical composition of the blend, the basic quality chemical parameters of the blends were subject to analysis. The results are given in Table 2. From results presented it is obvious that the samples of AC cigarette had highest concentration of nicotine, protein, total nitrogen and ash, but lowest amount of reducing sugars compared to other cigarette samples. However, the samples of RT cigarettes had the statistically significant lowest concentration of analyzed components except ashes.

Table 2. Chemical composition of experimental cigarettes and expanded stem

Cigarette	Content of compounds in tobacco blend (%)				
	Nicotine $\bar{x} \pm SD$	Protein $\bar{x} \pm SD$	Total nitrogen $\bar{x} \pm SD$	Reducing sugars $\bar{x} \pm SD$	Ash $\bar{x} \pm SD$
SC	1.7±0,03^{d}	7.2±0,07^{c}	2,6±0,01^{b}	10,7±0,04^{c}	17.1±0,03^{c}
AC	2.4±0,01^{a}	9.1±0,05^{a}	3,7±0,01^{a}	1.2±0,10^{e}	20.5±0,13^{a}
FC	1.9±0,02^{c}	6.0±0,02^{e}	2,1±0,01^{c}	16,6±0,01^{a}	16.9±0,04^{c}
RT	0.8±0,02^{f}	6,0±0,03^{e}	2,0±0,03^{c}	5.7±0,11^{d}	18.5±0,03^{b}
B_1	2.1±0,05^{b}	7.4±0,08^{b}	2,9±0,01^{b}	11.3±0,12^{b}	18.3±0,19^{b}
B_2	1.7±0,03^{d}	6.7±0,02^{d}	1,9±0,01^{d}	11.4±0,03^{b}	18.8±0,03^{b}
B_3	1.5±0,01^{e}	6.1±0,13^{e}	26±0,01^{b}	10,9±0,14^{c}	18.5±0,13^{b}
Expanded stem	0.53±0,01	4.11±0,02	1,90±0,01	13.84±0,3	24.32±0,2

Mean values with different letters in a column for each group are significantly from one another ($p < 0,05$).

According to results shown in Table 3, it can be concluded that were differences in TPM, nicotine and TAR content in cigarette smoke. Analysis of variance showed that tobacco blend composition has a significant impact on the content of the particulate phase cigarette smoke.

The lowest concentration of TPM, TAR and nicotine was determined in smoke of RT cigarettes. The content of TPM, TAR and nicotine concentration in blended cigarettes declines in the following order: $B_1 \rightarrow B_2 \rightarrow B_3$. The increasing amount of RT added in the mixtures proportionally decreases the content of TPM, TAR and nicotine. The addition of expanded stems in the tobacco mixture also has significant impact on the reduction of TPM, TAR and nicotine in tested cigarettes.

In this experiment, the hardness of cigarettes (67.95 ± 0.45%) was set as a fixed parameter in manufacturing of cigarettes. As a result of this, and due to a range of physical structures of used tobacco, there has been a change in the weight, puff number and draw resistance of the analyzed cigarettes (Table 4).

According to the data given in Table 4, RT cigarettes had the significantly lowest average weight, puff number and draw resistance. The weight difference was caused by the differences in the tobacco density. If we compare the blends, we can notice that cigarette B_1 has the highest average weight and puff number. The adding of 10% of recon and 15% of expanded stems to blend B_2 caused significantly increasing of tobacco filling power, which influenced decreasing of average cigarette weight, puff number and draw resistance.

Table 3. Composition particulate phase of mainstream cigarette smoke (mg/cig)

Cigarette	TPM $\bar{x} \pm$ SD	Nicotin $\bar{x} \pm$ SD	TAR $\bar{x} \pm$ SD
SC	22,6±0,04^{a}	1,13±0,04^{c}	18,48±0,11^{a}
AC	17,8±0,13^{c}	1,33±0,05^{a}	14,15±0,21^{c}
FC	19,4±0,08^{b}	1,26±0,02^{b}	15,63±0,16^{b}
RT	8,8±0,05^{f}	0,26±0,01^{f}	7,33±0,08^{f}
B_1	19,0±0,13^{b}	1,21±0,02^{b}	15,27±0,25^{b}
B_2	15,5±0,17^{d}	0,81±0,01^{d}	12,75±0,24^{d}
B_3	14,2±0,13^{e}	0,69±0,01^{e}	11,81±0,09^{e}

Mean values with different letters in a column for each group are significantly from one another (p < 0,05).

Table 4. Physical characteristic experimental cigarettes

Cigarette	Weight (g/cig.) $\bar{x} \pm$ SD	Puff number (per/cig.) $\bar{x} \pm$ SD	Draw resistance(mmH_2O) $\bar{x} \pm$ SD
SC	1.207±0.012^{ab}	12,1 0 ,115^{a}	120, 3± 0,015^{a}
AC	0.961±0.011^{bc}	7.6±0,057^{d}	114,4± 0,025^{c}
FC	1.098±0.001^{abc}	11.3±0,208^{b}	118,7± 0,075^{b}
RT	0.878±0.005^{c}	5.4±0,057^{e}	96,7±0,030^{f}
B_1	1,399±0.029^{a}	8,2±0,264^{c}	103,4±0,021^{e}
B_2	0.871± 0.001^{c}	7.6±0,173^{d}	96,7±0,036^{f}
B_3	0.917±0.001^{bc}	7.4±0,152^{d}	106,4±0.025^{d}

Mean values with different letters in a column for each group are significantly from one another (p < 0,05).

.

Results of investigations of individual and total PAHs in particulate phase of mainstream smoke calculated per cigarette are given in Table 5.

Table 5. The content of PAHs in smoke (TPM) of experimental cigarettes (ng/cig)

PAH	SC $\bar{x} \pm$ SD	AC $\bar{x} \pm$ SD	FC $\bar{x} \pm$ SD	RT $\bar{x} \pm$ SD	B_1 $\bar{x} \pm$ SD	B_2 $\bar{x} \pm$ SD	B_3 $\bar{x} \pm$ SD
B(a)P	24.03±0.7^{a}	11.73±0.9^{cd}	20.10±0.7^{b}	9.23±0.3^{d}	17.80±0.4^{b}	13.37±0.3^{c}	12.17±0.2^{cd}
B(a)A	44.17±2,5^{b}	19.71±0,5^{d}	56.42±0,9^{a}	11.70±3.6^{e}	32.93±0.4^{c}	29.85±4,5^{c}	26.65±5,4^{cd}
D(a.h)A	6.20±0.5^{ab}	3.40±0.6^{cd}	7.20±0.5^{a}	1.00±0.01^{e}	4.40±0.4^{bc}	1.83±0.1^{de}	1.43±0.1^{de}
B(b)F	16.63±1,2^{a}	11.90±0.8^{bc}	14.23±1,^{ab}	8.30±1,5^{d}	13.10±0.5^{bc}	11.70±0.7^{bc}	11.23±0.9^{c}
B(k)F	2.33±0,4^{a}	1.13±0.1^{b}	1.93±0.1^{ab}	1.73±0.5^{ab}	1.67±0.1^{ab}	2.07±0.1^{a}	1.63±0.1^{ab}
Flu	459.47±12,2^{a}	248.33±9,34^{c}	339.27±7.2^{b}	124.8±12,7^{e}	263.6±11,2^{c}	228.97±.4,1^{cd}	202.83±11,1^{d}

Pyr	221.7±3,84^{a}	94.87±0.7^{d}	209.0±3,9^{a}	65.5±1.3,8^{e}	151.8±4,4^{b}	120.57±2,2^{c}	96.3±9,7^{d}
Chry	79.43±0.8^{a}	25.7±2,4^{d}	40.27±3,5^{b}	17.5±0.7^{e}	34.87±0.7^{c}	25.6±1,95^{d}	23.7±0.5^{d}
Total PAHs	853.96±11,9^{a}	416.78±14,4^{de}	688.42±36,6^{b}	239.78±20,3^{f}	520.16±16,7^{c}	433.95±4,26^{d}	374.95±20,26^{d}

Mean values with different letters in a column for each group are significantly from one another ($p < 0,05$).

According to results shown in Table 5, it can be concluded that there were differences in individual and total PAHs content in particulate phase of mainstream smoke.
The significantly highest concentration of B(a)P was found in the smoke from SC cigarette, and the lowest in the smoke from RT cigarettes. Tobacco mixture B_1 also shows significant statistical differences regarding the content of B(a)P in relation to the tobacco mixtures B_2 and B_3. No significant statistical difference has been determined between B_3 and AC and B_1 and FC.
There are significant differences in the content of B(a)A in the smoke of the tested non-blended cigarettes, whereas among the blended cigarettes, the significant differences was not found.
The highest content of B(a)A is present in the smoke from FC cigarettes and the lowest in RT cigarettes. The reason for all of this was the fact that the amount of sugars in the FC tobacco was higher when compared with SC and AC tobacco. The analysis of data presented in Table 6 shows a strong positive correlation (r=0,80) between the concentration of B(a)A and sugars content in tobacco mixtures.

Table 6. Persons's coefficient of correlation of PAHs content in relation to the chemical and physical properties of cigarettes

Chemical and physical properties of cigarettes	Content of individual and total PAHs in cigarette smoke (ng/cigarette)								
	B(a)P	B(a)A	D(a,h)A	B(b)F	B(k)F	Flu	Pyr	Chry	Total PAH
Nicotine %	.293	.316	.446*	.468*	-.291	.400	.309	.160	.355
Protein %	.004	-.201	.116	.202	-.426	.189	-.055	.099	.081
Nitrogen protein (%)	-.003	-.203	.093	.204	-.415	.187	-.065	.091	.075
Totl nitrogen (%)	-.004	-.191	.137	.180	-.552**	.158	-.080	.079	.079
Reducing sugars %	.590**	.800**	.478*	.449*	.544*	.407	.669**	.329	.525*
Ash %	-.720**	-.728**	-.583**	-.521*	-.651**	-.557**	-.743**	-.607**	-.653**
Draw resistance/mm H_2O	.686**	.656**	.792**	.742**	.083	.812**	.713**	.697**	.783**
Weight/g	.636**	.459*	.625**	.549*	.190	.516*	.590**	.510*	.555**
Number puffing/cig	.928**	.904**	.873**	.900**	.455*	.953**	.967**	.866**	.975**

* Correlation is significant at the 0,05 level
** Correlation is significant at the 0,01 level

The lowest amounts of D(a,h)A, B(b)F, Flu, Pyr and Chry, also were detected in smoke from RT cigarettes. The smoke from FC cigarettes has the highest average concentration of D(a,h)A, which is not significantly differ from the average concentration of D(a,h)A in the smoke from SC cigarettes. The results showed that there was a medium strong positive correlation (Table 6) between the yield D(a,h)A in smoke and nicotine content (r=0,45), and strong negative correlation (r= - 0,58) with ash content in the blend.

Also, there was no significant difference between amounts of B(b)F in smoke FC and SC, and between AC, B_1 and B_2 cigarette. Increasing the share of expanded stems from 15 % to 30 % in blend B_3 contributed to the formation of lowest average amount of B(b)F, when compared to blend B_1 and B_2. Ding [25] also determined higher content of B(b)F in the smoke from SC (37,5 ng/cig.), FC (34,9 ng/cig.), AC (27,5 ng/cig), whereas in RT cigarettes lower content (14.6 ng/cig).

The analysis of data presented in Table 6 shows a strong negative correlation (r= -0.65) between the concentration of B(k)F and the average content of ashes, as well as amount of total nitrogen (r= -0,55) in the mixtures. For this reason, AC tobacco which contain the hihest amount of ashes and total nitrogen (Table 3) produces the lowest concentration of B(k)F in smoke. As the SC and FC tobaccos hava similar average content of ashes, they show a similar effect on the formation of B(k)F. RT tobacco has a higher average content of ashes in comparison to the SC and FC tobacco and produces a lower amount of B(k)F.

Content of Fly is much higher in the smoke from SC cigarettes compared to all other tested cigarettes. In smoke from B_3 cigarette was found significant low amount Flu, compared to smoke from B_1 and B_2 cigarettes. There are a strong negative correlation (Table 6) between content of Fly in smoke and ash content (r= -0,56) in the blend.

The highest amount of Pyr was found in the smoke from SC cigarettes, which did not significantly differ from the concentration of Pyr in the smoke from FC cigarettes. The significant difference has been determined in smoke from blended cigarette B_3 in relationship to smoke from B_1 and B_2. The strong positive correlation (Table 6) between reducing sugars in blend (r=0,67), and very strong negative correlation among ash content (r= -0,74) and Pyr content in smoke of cigarettes confirms obtained results.

Ding [25] point to the fact, that the use of SC and FC tobaccos give similar values of Chry (78.0 и 75.9 ng/cig). However, our results show that SC tobacco produces a significantly higher amount Chry.

The three representatives of this group of compounds, which according to IARC classification did not show any carcinogenic activity (fluorene, pyrene, chrysene) had the greatest share in the content of total PAHs. If we compare the non-blended cigarettes, we can conclude that the smoke from SC cigarettes contains the highest concentration of total PAHs.

Different amount of tobacco in mixtures B_1, B_2 and B_3, as well as the addition of expanded stems had an impact on the amount of PAHs formed in the smoke of blended cigarettes. The largest amount of PAHs was found in the smoke of blend B_1, which consisted of 60% FC, 30% AC and 10% SC tobacco, without recon and expanded stems. The lowest amount of PAHs was found in the smoke from the cigarettes B_3 with 10% recon and 30% expanded stems. In the previous analyses authors [26] determined 17 PAHs in mainstream smoke (MSS). The average yields of particulate phase PAHs were 1555 ng/cig, respectively. Ding [25] determined 14 PAHs in MSS with the range from 87 to 1598 ng/cig in particulate phase. In our analyses, the blended cigarettes revealer much lowers value. The difference of the results between this investigation and literatures may be associated with two possible reasons. First of all, we are determined only 8 individual PAHs. Second, we used pure tobaccos and blends without additives in our research.

The data in Table 6 show that total PAHs content in particulate faze cigarette smoke are statistically in a strong positive correlation with the puff number (r=0,97) and value of draw resistance (r=0,78) of analyzed cigarettes. Analysis of data presented in Table 7 reveals a strong positive correlation between the content of individual and total PAHs in smoke and the contents of TPMa (r = 0.90) and TARa (r=0.92) which is consistent with the literature data [26].

The significantly biggest puff number, draw resistance (Table 4) as well as highest concentracion of TPMa and TARa in smoke SC cigarette (Table 2) confirms the obtained results.

It is evident that significant positive correlation exists between B(a)P, B(a)A, D(a,h)A, B(b)F, fluorene, pyrene, chrysene and content of TPM and TAR. This is a further explanation of why the higest concentration of these PAHs was found in smoke from SC tobacco and mixture B_1. It is interesting that the amount of the produced B(k)F is not dependent on the content of TPMa, TARa and nicotine in smoke of experimental cigarettes.The data also shows statisticly significant positive corelation with average nicotine concentration (r = 0.65).

Table 7. Persons's coefficient of correlation of PAHs content in relation to the particulate phase composition

Content of PAHs in cigarette smoke	Content mainstream smoke of cigarettes (mg/cigarette)		
	TPM	Nicotin	TAR
B(a)P	.860**	.600**	.873**
B(a)A	.750**	.601**	.760**
D(a,h)A	.815**	.736**	.807**
B(b)F	.910**	.696**	.921**
B(k)F	.249	-.094	.283
Flu	.916**	.674**	.928**
Pyr	.856**	.629**	.867**
Chry	.792**	.457*	.814**
Total PAH	.902**	.655**	.916**

* Correlation is significant at the 0,05 level

CONCLUSION

The obtained results have showed that the percentage share of tobacco type, as well as the addition of recon and expanded stems in a cigarette blend had significant impact on the amount of total and individual PAHs in cigarette smoke. RT and SC tobaccos had the most evident impact.

The highest concentration of total PAHs was identified in the smoke from SC cigarettes and the lowest in RT cigarettes. Fluorene, pyrene and chrysene, which according to IARC classification did not show any carcinogenic activity, had the greatest share in the content of total PAHs.

Physical characteristics and chemical composition of the cigarettes and cigarettes smoke had a very strong impact on the PAHs production during the smoking process. A very strong positive correlation of PAHs with the puff numbers (r = 0.97) was determined in all analyzed cigarettes. There was a strong positive correlation (r = 0.78) between the content of PAHs and the draw resistance in all analyzed cigarettes. The concentration of total PAHs in cigarette smoke is statistically highly significant positive correlation with the average content of TPM (r = 0.90) and TAR (r = 0.92), as well as a statistically significant positive correlation with the average content of nicotine in tobacco smoke (r = 0.65).

REFERENCES

[1] A. Fawky, Cigarette Product Development, Tobacco Reporter/Speccomm. International, Raleigh, NC, USA, 2004.

[2] A. Rodgman, T. A. Perfetti, The chemical components of tobacco and tobacco smoke, CRC Press Taylor and Francis Group, Boca Raton, London, New York, 2009.

[3] A. Thielen, H. Klus, L. Müller, Tobacco smoke: unravelling a controversial subject, Experimental and Toxicologic Pathology 60 (2008) 141–156.

[4] A. Rodgman, T. A. Perfetti, The composition of cigarette smoke, A catalogue of the polycyclic aromatic hydrocarbonsm Beitr. Tabakforsch. Int. 22 (2006) 13-69.

[5] A. Rodgman, Studies of polycyclic aromatic hydrocarbons in cigarette mainstream smoke: Identification, tobacco precursors, control of levels, A review, Beitr. Tabakforsch. Int. 19 (2001) 361–379.

[6] A. M. Sehili, G. Lammel, Global fate and distribution of polycyclic aromatic hydrocarbons emitted from Europe and Russia, Atmospheric Environ. 41 (2007) 8301-8315.

[7] Lodovici, M.; Akpan, V.; Evangelisti, C.; Dolara, P., (2004). Sidestream tobacco smoke as the main predictor of exposure to polycyclic aromatic hydrocarbons. J. Appl. Toxicol., 24, 277-281.

[8] Kalaitzoglou, M.; Samara, C., (2006). Gas/particle partitioning and yield levels of polycyclic aromatic hydrocarbons and *n*-alkanes in the mainstream cigarette smoke of commercial cigarette brands. Food Chem. Toxicol., 44, 1432–1442.

[9] Yong, G.; Jin, Z.; Tong, H.; Yan, X.; Li, G.; Liu, S., (2006). Selective reduction of bulky polycyclic aromatic hydrocarbons from mainstream smoke of cigarettes by mesoporous materials. Microporous and Mesoporous Materials, 91, 238-243.

[10] Pérez-Gregorio, M. R.; García-Falcón, M. S.; Martínez-Carballo, E.; Simal-Gándara, J., (2010). Removal of polycyclic aromatic hydrocarbons from

organic solvents by ashes wastes. Journal of Hazardous Materials, 178, 273-281.

[11] Radojičić, V., Nikolić, M., Adnađević, B.: The influence of zeolite type added to the cigarette blend on the changes of pyrolitic temperatures, Hemijska Industrija, Vol 63 (5A), 579-583, 2009.

[12] Radojičić, V.; Alagić, S.; Adnadjević, B.; Maktouf, A. M., (2012). Effect of varied quantities of zeolite on the reduction of polycyclic aromatic hydrocarbons in tobacco smoke. African Journal of Biotechnology, 11, 10041-10047.

[13] ISO Standard 15152, (2003). Tobacco **-** Determination of the Content of Total Alkaloids as Nicotine **-** Continuous-flow Analysis Method.

[14] ISO Standard 15154, (2003). Tobacco **-** Determination of the Content of Reducing Carbohydrates **-** Continuous-flow Analysis Method.

[15] Bremner, J. M.; Mulvaney, C. S., (1982). Nitrogen-total in: Methods of Soil Analysis. Part 2, Chemical and microbiological properties, Madison Wis, ASA, Monograph 9, 2nd, 595–624.

[16] AOAC Association of Official Analytical Chemists, (1997). Official Methods of Analysis of the Association of Official Analytical Chemists, 14rd. Ed. Arlington (VA), AOAC.

[17] ISO Standard 6565, (2002). Tobacco and tobacco products **-** Draw resistance of cigarettes and pressure drop of filter rods **-** Standard conditions and measurement.

[18] ISO Standard 3402, (1999). Tobacco and tobacco products - Atmosphere for conditioning and testing.

[19] ISO Standard 3308, (2000). Routine analytical cigarette-smoking machine - Definitions and standard conditions.

[20] ISO Standard 10315, (2000). Cigarettes - Determination of nicotine in smoke condensates - Gas-chromatographic method.

[21] ISO Standard 10362-1, (1991). Cigarettes - Determination of water in smoke condensates - Part 1: Gas-chromatographic method.

[22] ISO Standard 4387, (2000). Cigarettes - Determination of Total and Nicotine-Free Dry Particulate Matter Using a Routine Analytical Smoking Machine.

[23] Radojičić, V.; Alagić, S.; Adnadjević, B.; Maktouf, A. M., (2012). Effect of varied quantities of zeolite on the reduction of polycyclic aromatic hydrocarbons in tobacco smoke. African Journal of Biotechnology, 11, 10041-10047.

[24] De Coster. J. (2004): Data Analysis in SPSS. CA: Academic Press, San Diego

[25] Ding, Y. S.; Trommel, J. S.; Yan, X. J.; Ashley, D.; Watson, C. H., (2005). Determination of 14 polycyclic aromatic hydrocarbons in mainstream smoke from domestic cigarettes. Environ Sci Technol., 39 (2), 471–478.

[26] Lu, H.; Zhu, L., (2007). Pollution patterns of polycyclic aromatic hydrocarbons in tobacco smoke. Journal of Hazardous Materials, 139 (2), 193-198.

BIOLOŠKA AKTIVNOST EKSTRAKATA VRSTE *Juniperus communis* L. *

BIOLOGICAL ACTIVITY OF *Juniperus communis* L. EXTRACTS

Irma Mahmutović[1], Sabina Dahija[1], Renata Bešta-Gajević[1], Erna Karalija[*1]

Original scientific paper

Rezime

Juniperus communis L. je biljka koja se koristi u narodnoj medicini, osobito njeni češeri, u tretmanu brojnih infekcija, te kao začin i aroma u pripremi određenih pića. U ovom istraživanju izvršeno je određivanje ukupnog sadržaja fenola i flavonoida, antioksidativne aktivnosti, te testiranje antimikrobnih svojstava metanolnih ekstrakata listova i češera obične borovice. Ekstrakcija je vršena 80% metanolom iz ovogodišnjih i prošlogodišnjih listova sa ženskih i muških individua, te češera ženskih individua. Ukupni sadržaj fenola je bio statistički značajno veći u grančicama muških individua, a isto je potvrđeno i za sadržaj flavonoida. Reducirajuća moć metanolnih ekstrakata je bila značajno veća u odnosu na korištenu kontrolu (α-pinol), a redukcija DPPH radikala se kretala od 91 do 94%. Antimikrobna aktivnost metanolnih ekstrakata je određena disk-difuzionom metodom. U ovom istraživanju korištene su dvije vrste Gram-pozitivnih bakterija: *Staphylococcus aureus* ATCC 6538 i *Bacillus subtilis* ATCC 6633, dvije vrste Gram-negativnih bakterija: *Escherichia coli* ATCC 8739 i *Pseudomonas aeruginosa* ATCC 9027, te gljivica *Candida albicans*. Metanolni ekstrakti borovice su pokazali antibakterijsko djelovanje na testirane sojeve u različitom stepenu. Gram-negativna vrsta *P. aeruginosa* se pokazala najsenzitivnijim sojem u poređenju sa referentnim antibiotikom ampicilinom. Rezultati su pokazali da *Juniperus communis* ima veliki antioksidativni i antimikrobni potencijal, osobito listovi muških individua.

Ključne riječi: *antimikrobno dejstvo, antioksidativno dejstvo, češeri borovice, listovi borovice*

Summary

Juniperus communis L. is one of the plants used in folk medicine, especially juniper berries, for the treatment of many infections, and as a spice and flavour in preparation of some drinks. Total phenol and flavonoid content, DPPH radical scavenging activity

---

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Faculty of Natural Sciences and Mathematics, University of Sarajevo, Zmaja od Bosne 33-35, 71 000 Sarajevo, Bosnia and Herzegovina
* Corresponding author; erna.k@pmf.unsa.ba

and antimicrobial properties of methanol extracts of leaves and berries were determined. Extraction, using 80% methanol, was done from female and male plants young and old shoots, and berries from female plants. Total phenol content was statistically higher in shoots of male plants; the same was recorded for flavonoid content. The reducing power of methanol extracts was statistically higher than used control (α-pinol), and reduction of DPPH radical ranged from 91 up to 94%. Antimicrobial activity was determined using paper disc diffusion method. In this research two Gram-positive bacteria *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, two Gram-negative bacteria *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, and yeast *Candida albicans* were used. Juniper methanol extracts exhibited antibacterial activity against tested strains in variable degree. Gram-negative *P. aeruginosa* was the most sensitive tested strain compared to reference ampicillin antibiotic. The results showed that *Juniperus communis* has a great antioxidant and antimicrobial potential, especially leaves of the male plants.

Key words: *antimicrobial activity, antioxidant activity, juniper berries, juniper leaves*

INTRODUCTION

Mankind was always dependent on plants, because they provide the components necessary for its existence (Simpson & Ogorzaly, 1995). The use of plants for the treatment of the disease has been known since ancient times, and even today many plant products are traditionally used in medicine (Dhawan, 2003; Sati & Joshi, 2010). Scientific validation of medicinal plants is ensured through various phyto-pharmacological studies that define plant components. However, it is estimated that of 250.000 to 400.000 plant species, only 6% have been explored in terms of biological activity, and 15% have been explored in terms of phytochemistry. This demonstrates the need for a phyto-pharmacological evaluation of herbal drugs (Goyal *et al*., 2007). Over the past decade, the clinical efficiency of many synthetic antibiotics has been significantly reduced due to the emergence of multiresistant pathogens, which directed investigations towards the finding a new sources of antimicrobial substances (Eldeen *et al*., 2005).

According to Stace (2010), genus *Juniperus* L. (Cupressaceae) include between 50 and 67 species, depending on taxonomic viewpoint. In terms of life form, these species are mainly shrubs or trees, and are widely distributed throughout the Northern Hemisphere. This genus is represented in Bosnia and Herzegovina by six species: *Juniperus communis* L. (common juniper), *J. oxycedrus* L. (prickly juniper), *J. macrocarpa* S.S. (large-fruited juniper), *J. nana* Willd. (dwarf juniper), *J. phoenicea* L. (Phoenicean juniper), and *J. sabina* L. (savin juniper) (Šilić, 2005).

J. communis is a dioecious and evergreen prostrate shrub, very variable in form, and characterized with wide ecological valence. *Juniperus communis* is considered as an important medicinal plant used in traditional medicine for centuries (Sela *et al*., 2013). Their berries and leaves have been used for a long time to treat different disease: to lower hypertensia, and as an antidiabetic and diuretic (De Medina *et al*., 1994; Banerjee *et al*., 2012); externally for rheumatic symptoms and internally for many gynecological conditions (Emami *et al*., 2007; Sati & Joshi, 2010). Juniper oil is used in the pharmaceutical and food industries and parfumery, as well as in cosmetics (Haziri *et al*., 2013). Juniper berries (female cones) are used commercially for the preparations of essential oils, gin and as a spice. Furthermore, berries has digestive, stomachic, and anthiureumatic proeperties (Pepeljnjak *et al*., 2005). In terms of antimicrobial potential, activity of *J. communis* has been proven against the cca. 40 different species of fungi, viruses and bacteria, including some resistant clinical strains (Mahmutović & Muratović, 2014).

There is lack of data regarding analysis of methanol leaf extracts against microorganisms and antioxidant potential. For this reason, the aim of the presented study was to evaluate the differences between the effectivness of berries and leaf extracts regarding antioxidant and antimicrobial activity. Furthermore we evaluated sex and maturity differences in chemical composition, antioxidant and antimicrobial potential of the extracts.

MATERIAL AND METHODS

Plant material

The plant material (leaves and berries) of *J. communis* was collected in June 2017 on the mountain Ozren near Sarajevo, Bosnia and Herzegovina. Separation of plant material into old and one year sprouts, berries and female and male plants was carried out in Laboratory for Plant Physiology, Faculty of Natural Sciences and Mathematics, University of Sarajevo. Separated plant material was dried in the shade at room temperature in ventilated room, and later deposited at +4 °C until use.

Extract preparation

Dried plant material was separated into: female and male plants; and further separated into old leaves (♀ and ♂ OL); this year sprouts (♀ and ♂ TYS) and immature green berries (BER; for female plants). All material was grounded to fine dust and then extracted twice using 80% methanol. Extraction was performed in ultrasonic bath for 30 minutes. Both extracts were combined to obtain final extracts which were diluted to final concentration of 1 mg/mL for phenolics, antioxidant and antimicrobial potential analysis. All extracts were stored at +4 °C until use.

Total phenol and flavonoid content

Analysis of total phenol content in methanol extracts was performed according to Wolfe *et al*. (2003), using the Folin-Ciocalteu reagent and gallic acid (GA) as standard. Analysis of total flavonoid content was performed according to Ordonez *et al*. (2006), with catechin (CE) as standard. All results were expressed as mg of used standard equivalent per mg of extract.

Antioxidant potential

Antioxidant activity was evaluated using the same extracts according to the DPPH (2,2-diphenyl-1-picrylhydrazyl) method described by Meda *et al*. (2005). Antioxidant potential (AP) was evaluated according to the absorbance change after 30 minutes. Antioxidant potential was calculated as a radical inhibition (%) according to the formula:

$$AP\% = [(A0-A30)/A0]*100$$

Where:
AP% - antioxidant potential
A0 - sample absorbance reading at 0 seconds
A30 - sample absorbance reading after 30 minutes
As a standard α-pinol was used in same concentrations as extracts (1 mg/mL)

Antimicrobial potential

In this research two strains of Gram-positive bacteria *Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC 6633, and two strains of Gram-negative bacteria *Escherichia coli ATCC 8739, Pseudomonas aeruginosa ATCC 9027*, and yeast *Candida albicans* were used. Bacterial strains were cultured overnight at 37 °C on Mueller Hinton broth and adjusted in sterile saline solution to a final density of 0.5 McFarland standard (1.5 × 10^8 CFU/mL). *Candida albicans* was grown overnight at 37 °C in Sabouraud Dextrose Agar, and inoculum was prepared in sterile saline solution and adjusted to 0.5 Mc Farland scale. In order to evaluate the antimicrobial effect of juniper methanol extracts, the Kirby-Bauer disk diffusion method was applied according to slightly modified National Commitee for Clinical laboratory standards, 2001. After applying extracts the samples were incubated at 37 °C for 24-48 hours. The diameters of the inhibition zones were measured in millimeters. All tests were performed in triplicate.

RESULTS AND DISCUSSION

Total phenol and flavonoid content

The total amounts of phenolic compounds in all investigated samples vary in limits from 271.492 to 544.088 mg GA/g DW. Total phenol and flavonoid content in *J. communis* methanol extracts were determined by UV-Vis spectrometric measurements. The highest content of total phenols was recorded for old leaves of male plants; while lowest value was recorded for berries. After Newman-Keuls analysis statistically significant differences were determined between analysed extracts (Table 1). Total flavonoid content varied between 29.229 and 49.094 mg CE/g DW. Statistically significant differences were determined between male and female individuals, where male plants contained higher amounts of flavonoids. It was interesting that immature green berries contained only small amount of flavonoids (Table 1).

Determined differences between analysed samples are probably the result of genetic or environmental factors. It is known that final composition of plant extracts (phenolic and flavonoid content) is determined both genetically and by environmental factors (Miceli *et al*., 2009).

In dioecious plants there is often difference between growth and defence of female and male plants. Usually males allocate more resources to growth while females to secondary metabolites synthesis (Herms & Mattson, 1992; Massei *et al*., 2006) but there is also other cases when females invest less than males in secondary metabolites (Massei *et al*., 2006). In our study males had higher metabolite content than females, although no signs of herbivory were observed in either sex.

The lack of herbivory could be associated the insecticidal and to the feeding inhibition properties of some terpenoids found in juniper leaves (Massei *et al*., 2006). The sex-related difference in resource allocation found in our study is in accordance with Mole's (1994) resource allocation model. According to this model plants can allocate resources to different function without a decrease of chemical defences and vice versa (Karban & Baldwin, 1997).

Lower values in secondary metabolites content found in female tries could be explained by the fact that female tries produce long-lived fruits and favour reproduction at the expense of growth and defence (Obeso, 2002).

Table 1. Total phenolics, flavonoids and antioxidant activity of juniper extracts according to sex and maturity

Sample/standard	**Total phenolics (mgGA/gDW)**	**Total flavonoids (mgCE/gDW)**	**Antioxidant potential (%)**
♂ **TYS**	486.218 ± 11.148[b]	49.094 ± 2.829[a]	94.366 ± 0.101[a]
♂ **OL**	544.088 ± 22.191[a]	48.061 ± 2.065[a]	94.203 ± 0.029[a]
♀ **TYS**	452.788 ± 22.143[b]	34.717 ± 0.553[c]	92.811 ± 0.533[a]
♀ **OL**	390.888 ± 14.912[c]	40.223 ± 1.569[b]	93.162 ± 0.351[a]
BER	271.492 ± 21.305[d]	29.229 ± 0.185[d]	91.993 ± 0.601[a]
α-Pinol			18.351 ± 0.001[b]

♂ OL; TYS - male old leaves and this year sprouts respectively; ♀ OL; TYS - female old leaves and this year sprouts respectively; BER - berries. In each column, mean ± SD followed by the same letter in subscript was not significantly different (p≤0.01) according to the Student-Newman-Keuls test

Antioxidant potential

The reducing power of *J. communis* extracts are shown in Table 1. All extracts had statistically higher reducing power than used standard, α-pinol. No statistical differences between analysed extracts were observed (Table 1). The DPPH exposed to antioxidant compounds changes its purple colour to yellow. The degree of discoloration of DPPH indicates the scavenging potential of the antioxidant extract (Siriwardhana & Shahidi, 2002; Moein & Moein, 2010). The most important antioxidant compounds are phenolics especially flavonoids. In our study high amounts of phenolics could be responsible for high antioxidant potential, reaching 94%. It is previously reported that antioxidant activity correlates with the phenolic content (Moein *et al*., 2008; Moein & Moein, 2010).

Antimicrobial potential

In this investigation, antimicrobial activity of methanol extracts obtained from the leaves and berries of *J. communis* was tested against five microorganisms. The results indicated that all extracts showed activity with varying degrees of growth inhibition, depending on the microbial species, as well as on the extract type (Table 2). The extract obtained from male old leaves was the most effective in terms of antibacterial activity, with highest inhibition on Gram-negative bacterial strain of *P. aeruginosa*. These results were interesting, since the *P. aeruginosa* is versatile pathogen well known by its multidrug-resistance (Hirsch & Tam, 2010). Inhibition zones on *P. aeruginosa* caused by standard antibiotic Ampicillin (10 μg), were slightly wider (14 mm), compared to those made by investigated extract, ranging from 6 to 11 mm. Considering the fact that Gram-negative bacteria have an outer lipopolysaccharide wall, which makes them more resistant to antibacterial substances, these findings of significant natural inhibition are promising. Extract obtained from male old leaves had also very effective antibacterial action on *E. coli* with average inhibition zones of 8.33 mm.

Extract made from this year male sprouts showed high antibacterial potential, especially on Gram-positive bacteria *B. subtilis* and *S. aureus*. Detected inhibition zones on *B. subtillis* were 9.33 mm, and 7 mm for *S. aureus*. Comparing the extracts obtained from female juniper individuals, higher antibacterial activity was observed for extracts from old leaves, with the most prominent results against *E. coli* (inhibition zones 8 mm). This year sprouts extract of female plant had lower antibacterial activity, with no overall effect on *B. subtillis*. Elmhdwi *et al*. (2015) reported that methanol extracts made from closely related *J. phoenicea* have significant antibacterial potential due to abundance of the α-pinene and overall chemical constituents. In this study, methanol extracts made from juniper leaves were more efficient against all tested microorganisms compared to the juniper berry extract, which opens the possibility of wider use of *J. communis* leaves in preparation of natural antibacterial agents. Investigated yeast *C. albicans*, was the most sensitive to the old leaves extract, while juniper berry extract did not show any antifungal potential. These results provide the possibility of using *J. communis* leaves extract in the treatment of fungal infections.

Significantly higher amounts of flavonoids, important antimicrobial agents (Cushnie & Lamb, 2005), were detected in male juniper individuals, which is in correlation to the results generated through antimicrobial tests. Considering all obtained results, it is obvious that leaves extracts from male individuals have higher antimicrobial activity (Table 2).

Table 2. Antimicrobial activity of *Juniperus communis* methanol extracts

Sample	*Escherichia coli*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Staphylococcus aureus*	*Candida albicans*
♂ **TYS**		5.33±4.72	9.33±0.57	7.00±0.00	3.00±5.19
♂ **OL**	8.33±0.57	10.33±0.57	6.67±0.57	6.67±0.57	7.00±0.00
♀ **TYS**	7.67±1.52	6.00±0.00		6.33±0.57	6.67±0.57
♀ **OL**	8.00±1.00	6.67±0.57	6.67±0.57	6.67±0.57	7.00±0.00
BER	6.67±0.57	7.00±0.00	6.67±0.57	7.00±1.00	

♂ OL; TYS - male old leaves and this year sprouts respectively; ♀ OL; TYS - female old leaves and this year sprouts respectively; BER - berries.

CONCLUSIONS

Common juniper is well known medicinal plant, but studies regarding its biological activity are predominantly oriented toward essential oil features, especially the oil isolated from berries. This investigation highlighted significant biological activity of juniper methanol extracts, both from leaves and from berries. Furthermore, maturity and sex differences of extracts were noted, where extract obtained from old male plants showed highest biological activity. Results generated from analysis of antioxidant activity correlated with results regarding antimicrobial potential, where detected high flavonoid content in old male leaves showed high antibacterial activity against multi-resistant pathogens. This investigation confirmed previously known medicinal properties of common juniper, but also opened the possibility of wider use of its methanol extracts as natural and safe preparation in treating infection caused by microorganisms.

REFERENCES

Banerjee, S., Mukherjee, A. & Chatterjee, T. K. (2012). Evaluation of analgesic activities of methanolic extract of medicinal plant *Juniperus communis* Linn. *International Journal of Pharmacy and Pharmaceutical Sciences*, *4*(5), 547-550.

Cushnie, T. P. T. & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents, 26*(5), 343-356.

De Medina, F. S., Gamez, M. J., Jimenez, I., Jimenez, J., Osuna, J. I. & Zaruelo, A. (1994). Hypoglycemic activity of Juniper ‘‘berries’’. *Planta Medica*, *60*(3), 197-200.

Dhawan, B. N. (2003). WHO monographs on selected medicinal plants. *Indian Journal of Medical Research, 3*, 97-98.

Eldeen, I. M. S., Elgorashi, E. E. & Staden, J. V. (2005). Antibacterial, anti-inflammatory, anti-cholinesterase and mutagenic effects of extracts obtained from some trees used in South African traditional medicine. *Journal of Ethnopharmacology, 102*(3), 457-464.

Elmhdwi, M. F., Attitalla, I. H., Ali Khan, B. & Menaa, F. (2015). Evaluation of Antibacterial Activity and Antioxidant Potential of Different Extracts from the Leaves of *Juniperus phoenicea*. *Plant Pathology & Microbiology, 6*(9), 300.

Emami, S. A., Javadi, B. & Hassanzadeh, M. K. (2007). Antioxidant Activity of the Essential Oils of Different Parts of *Juniperus communis*. subsp. *hemisphaerica* and *Juniperus oblonga*. *Pharmaceutical Biology, 45*(10), 769-776.

Goyal, B. R., Goyal, R. K. & Mehta, A. A. (2007). Phyto-pharmacology of *Achyranthes aspera*: A Review. *Pharmacognosy Reviews, 1*.

Haziri, A., Faiku, F., Mehmeti, A., Govori, S., Abazi, S., Daci, M., Haziri, I., Bytyqi-Damoni, A. & Mele, A. (2013). Antimicrobial properties of the essential oil of *Juniperus communis* (L.) growing wild in east part of Kosovo. *American Journal of Pharmacology and Toxicology, 8*(3), 128-133.

Herms, D. A. & Mattson, W. J. (1992). The dilemma of plants: to grow or defend. *The quarterly review of biology, 67*(3), 283-335.

Hirsch, E. B. & Tam, V. H. (2010). Impact of multidrug-resistant *Pseudomonas aeruginosa* infection on patient outcomes. *Expert Review of Pharmacoeconomics & Outcomes Research, 10*(4), 441-451.

Karban, R. & Baldwin, I. T. (1997). Induced Responses to Herbivory. Chicago: The University of Chicago Press.

Mahmutović, I. & Muratović, E. (2014). Antimicrobial potential of forest plants from different areas of Bosnia and Herzegovina; *Jedanaesti simpozijum o zaštiti bilja u BiH*, Društvo za zaštitu bilja u BiH, Teslić, Bosna i Hercegovina; Abstracts: 82.

Massei, G., Watkins, R. & Hartley, S. E. (2006). Sex-related growth and secondary compounds in *Juniperus oxycedrus macrocarpa*. *Acta Oecologica, 29*(2), 135-140.

Meda, A., Lamien, C. E., Romito, M., Millogo, J. & Nacoulma, O. G. (2005) Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chemistry, 91*(3), 571-577

Miceli, N., Trovato, A., Dugo, P., Cacciola, F., Donato, P., Marino, A., Bellinghieri, V., La Barbera, T. M., Güvenç, A. & Taviano, M. F. (2009). Comparative analysis of flavonoid profile, antioxidant and antimicrobial activity of the berries of *Juniperus communis* L. var. *communis* and *Juniperus communis* L. var. *saxatilis* Pall. from Turkey. *Journal of agricultural and food chemistry*, *57*(15), 6570-6577.

Moein, M. R., Moein, S. & Ahmadizadeh, S. (2008). Radical scavenging and reducing power of *Salvia mirzayanii* subfractions. *Molecules*, *13*(11), 2804-2813.

Moein, M. & Moein, S. (2010). Antioxidant Activities and Phenolic content of Juniperus excelsa extract. *Iranian Journal of Pharmaceutical Sciences*, *6*(2), 133-140.

Mole, S. (1994). Trade-offs and constraints in plant-herbivore defense theory: a life history perspective. *Oikos*, *71*(1), 3-12.

National Committee for Clinical Laboratory Standard. (2001). In *Performance standards for anti-microbial susceptibility testing*: 11th informational supplement. Document M100-S11, Wayne, PA.

Obeso, J. R. (2002). The cost of reproduction in plants. *New Phytologist*, *155*(3), 321-348.

Ordonez, A. A. L., Gomez, J. D. & Vattuone, M. A. (2006). Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. *Food Chemistry*, *97*(3), 452-458.

Pepeljnjak, S., Kosalec, I., Kalođera, Z. & Blažević, N. (2005). Antimicrobial activity of juniper berry essential oil (*Juniperus communis* L., Cupresaceae). *Acta Pharmaceutica*, *55*(4), 417-422.

Sati, S. C. & Joshi, S. (2010). Antibacterial potential of leaf extracts of *Juniperus communis* L. from Kumaun Himalaya. *African Journal of Microbiology Research* *4*(12), 1291-1294.

Sela, F., Karapandzova, M., Stefkov, G., Cvetkovikj, I., Trajkovska-Dokik, E., Kaftandzieva, A. & Kulevanova, S. (2013). Chemical composition and antimicrobial activity of leaves essential oil of *Juniperus communis* (Cupressaceae) grown in Republic of Macedonia. *Macedonian pharmaceutial bulletin*, *59*(1,2), 23-32.

Simpson, B. B. & Ogorzaly, M. C. (1995). *Economic botany*. *Plants in Our World*. (2nd ed.). New York: McGraw-Hill, Inc.

Siriwardhana, S. & Shahidi, F. (2002). Antiradical activity of extracts of almond and its by-products. *Journal of the American Oil Chemists' Society*, *79*(9), 903-908.

Stace, C. (2010). *New flora of the British Isles*. Cambridge: Cambridge University Press.

Šilić, Č. (2005). *Atlas dendroflore (drveće i grmlje) Bosne i Hercegovine*. Čitluk: Matica Hrvatska.

Wolfe, K., Wu, X., Liu, R. H. (2003). Antioxidant activity of apple peels. *Journal of Agricultural and Food Chemistry*, *51*(3), 609-614.

KVANTITATIVNA ANALIZA SEKUNDARNIH METABOLITA VRSTE *Hypericum perforatum* PORIJEKLOM SA RAZLIČITIH LOKALITETA U BOSNI I HERCEGOVINI*

QUANTITATIVE ANALYSIS OF SECONDARY METABOLITES IN *Hypericum perforatum*, ORIGINATING FROM DIFFERENT LOCALITIES IN BOSNIA AND HERZEGOVINA

Azra Karadža[1], Erna Karalija[1], Danijela Vidic[1], Adisa Parić[1]

Original scientific paper

Rezime

Hypericum perforatum L. (Gospina trava, kantarion) bila je korištena stoljećima za liječenje opekotina, modrica, oticanja, upala i anksioznosti, kao i bakterijskih i virusnih infekcija. Cilj ovog istraživanja bio je ispitati sadržaj ukupnih fenola i flavonoida kantariona (*Hypericum perforatum* L.) čije su populacije porijeklom sa različitih lokaliteta u Bosni i Hercegovini. Sadržaj ukupnih fenola i flavonoida je jako varirao zaviosno od dijela biljke i od lokaliteta. Sadržaj ukupnih fenola je varirao od 10.55 do 26.72 mg GAE/gDW u ekstraktima cvjetova sa lokaliteta Dejčići i Trebević, a najveći sadržaj u ekstraktima listova je zabilježen na lokalitetu Trebević (24.07 mg GAE/gDW). Generalno, sadržaj flavonoida bio je veći u ekstraktima listova u odnosu na cvjetove, a najveći sadržaj zabilježen je na lokalitetu Breka (117.62 mg CE/gDW). Uporedni prikaz sadržaja ukupnih fenola i flavonoida u cvjetovima i listovima pokazao je veći sadržaj ovih supstanci u cvjetovima nego listovima. Rezultati su pokazali pozitivnu korelaciju između količine ukupnih fenola i flavonoida u svim metanolnim ekstraktima (list i cvijet). Studija je utvrdila da promjena okolišnih faktora može uticati na sintezu ukupnih fenola i flavonoida u *Hypericum perforatum* L., ali da nije jedini faktor.

Ključne riječi: *Hypericum perforatum, fenoli, flavonoidi, okolišni faktori*

Summary

Hypericum perforatum L. (St. John's wort) has been used for centuries in the treatment of burns, bruises, swelling, inflammation, bacterial and viral infections. The aim of this study was determination of total phenolics and total flavonoids content in *Hypericum perforatum* in populations originating from different locations in Bosnia and Herzegovina. Methanol extracts of flowers and leaves were obtained by the means

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Faculty of Science, University of Sarajevo, Zmaja od Bosne 33-35, 71 000 Sarajevo, Bosnia and Herzegovina

of ultrasound extraction. The total phenolics and flavonoids content varied with plant part and locality. Total phenolics and flavonoids content in the flowers extracts was higher than in the leaves extracts. Total phenolic content varies from 10.55 to 26.72 mg GAE/gDW for flower extracts from Dejčići and Trebević population respectively, and highest content in leaves extract were also find for Trebević population 24.07 mg GAE/gDW. Content of flavonoids were higher in leaves than in flower extracts and the highest content was 117.62 mg CE/gDW for sample from Breka population. A positive correlation between the amount of the total phenolics and total flavonoids in all methanol extracts was observed. The study confirmed that change in environmental factors may affect the synthesis of phenols and flavonoids in *Hypericum perforatum*, but also that genetic diversity may contribute to this variation.

Key words: *Hypericum perforatum, phenolics, flavonoids, environmental factors*

INTRODUCTION

Hypericum is a genus with more than 400 species that are widely distributed in temperate regions in all parts of the world and they are traditionally used to treat eczema, burns, trauma, rheumatism, neuralgia, hysteria, gastroenteritis, gastric ulcers, inflammation of the bronchi and the urogenital tract, urinary bladder irritation, colds, diabetes mellitus, and dyspepsia (Maleš *et al*., 2006; Pirbalouti *et al.*, 2011). This genus is native in Europe, Asia, and North Africa. Four subspecies of *Hypericum perforatum* namely: *Hypericum perforatum* subsp. *angustifolium*, *Hypericum perforatum* subsp. *latifolium*, *Hypericum perforatum* subsp. *vulgare* and *Hypericum perforatum* subsp. *veronense* exist in the southeastern Europe (Glišić *et al*., 2006). *Hypericum perforatum* L. is known for centuries; perennial, medicinal plant, also known as St. John's wort. This species has been reported to have antibacterial, antiviral, anti-inflammatory and anti-cancer properties, and its international popularity is due primarily to the treatment of psychological disorders, depression disorders or nervousness and wound healing (Odabas *et al.*, 2009). Interest for the study and isolation of secondary metabolites of this genus is growing. In the past, the secondary metabolites were observed as waste products of primary metabolism. Today it is known that such opinions were wrong and compounds are very important in defence against herbivores, ultraviolet rays or pathogen attacks (Bennett & Wallsgrove, 1994; Pandey & Rizvi, 2009). In recent years, plant-derived natural antioxidants, phenols and flavonoids, have been used frequently. Most of the beneficial health effects have been attributed to their antioxidant capabilities. The cells are continuously exposed to exogenous and endogenous oxidative stress, therefore, the production of free radicals is part of the metabolism. Free radicals can cause damage to cells and tissues in several ways: by damaging molecular and cellular components, by activating certain signalling pathways, by modification of gene expression and enzyme activity and interruption of the normal cellular repair mechanisms. Some phenols are capable of pairing electrons to free radicals and have the ability to bind pro-oxidant metals

(Fe^{2+}, Cu^{2+}). Moreover, the effects of some phenols are related to the increase in activity of antioxidant enzymes and the induction of synthesis of antioxidant proteins (Walter & Marchesan 2011). The aim of the present study was to determine the content of total phenols and flavonoids in the leaves and flowers of *Hypericum perforatum*, in relation to different collection localities in Bosnia and Herzegovina.

MATERIAL AND METHODS

Plant material

Leaves and flowers of *Hypericum perforatum* L., collected in the period July/August 2014 in the flowering phenophase, have been used for the purpose of the experimental part of this research. The plant material has been collected at 16 different localities (Figure 1).

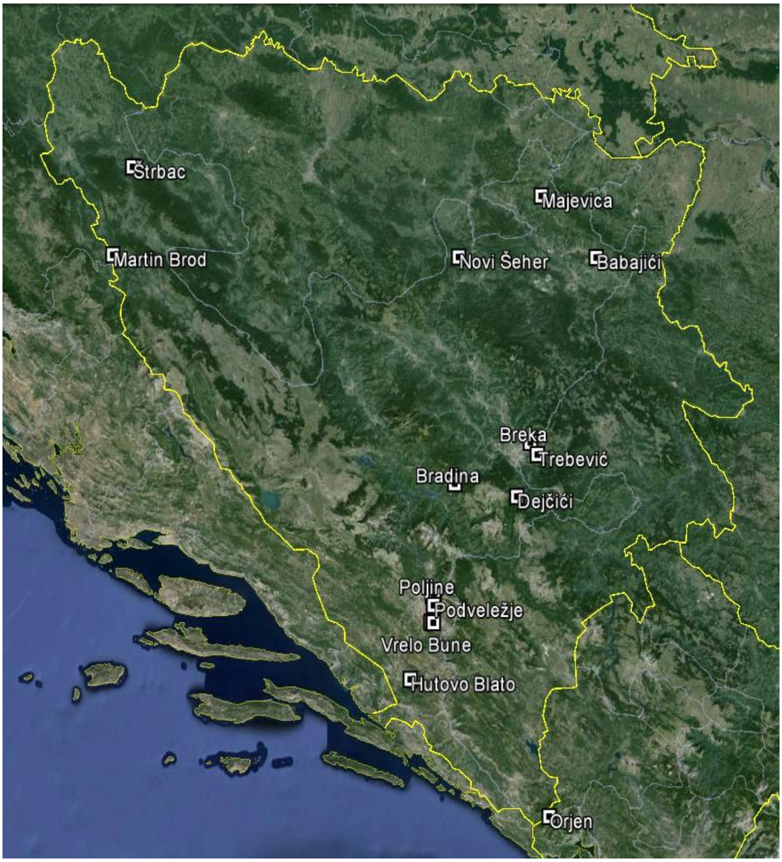


Figure 1. Satellite BiH map, study locations and their altitudes of the tested *H. perforatum*

(1) Štrbac (290m); (2) Martin Brod (330m); (3) Majevica (780 m); (4) Novi Šeher (235 m); (5) Babajići (275 m); (6) Breka (660 m); (7) Trebević (1180m); (8) Bradina (810 m); (9) Dejčići (936 m); (10) Poljine (780 m); (11) Velež (1064 m); (12) Podveležje (680 m); (13) Vrelo Bune (107 m); (14) Blagaj (320 m); (15) Hutovo Blato (50 m); (16) Orjen (1300 m).

Determination of individuals was conducted by Prof. dr. Edina Muratović, and Voucher specimens (No. LRPER 347-363) were deposited in the Laboratory for research and protection of the endemic gene pool. The rest of the plant material was separated into floral and leaves tissues and have been separately dried in a well ventilated room, protected from light, at the room temperature, for 7 days.

Preparation of plant extract

Air-dried plant material was extracted in 10 ml of 80% methanol by ultrasonication at room temperature for 60 min in an ultrasonic bath. The prepared extracts were filtered through a paper filter and kept in a refrigerator until analysis.

Analysis of total phenolic content

Total phenols were determined using a modified method of Wolfe *et al*. (2003), using the Folin-Ciocalteu reagent. The reaction mixture was prepared by mixing 10µL of methanol extract, 10 µl of methanol, 1580 µl of distilled water, 100 µl of Folin-Ciocalteu reagent and 300 µl of 7.5% aqueous Na_2CO_3 (w/v). After incubation, the spectrophotometric reading of absorbance was performed at a wavelength of 765 nm (Perkin Elmer, Lambda 25, UV/VIS Spectrophotometer) compared to the blank. The total phenolic contents in the methanol extract of flowers and leaves of *Hypericum perforatum* were quantified with the help of a standard calibration curve (y = 0,0118x; R^2 = 0.9955), and are reported as gallic acid equivalent (mg GAE/gDW). All measurements were performed in three replications.

Analysis of total flavonoid content

Analysis of total flavonoid content in the tested samples was carried out using a modified method of Ordonez *et al*. (2006) with catechin as standard. The reaction mixture contained: 10 µl aliquots of the appropriate methanol extract, 10 µl of methanol, 105 µl of distilled water, 25 µl of 10% aqueous $AlCl_3$ (w/v), 25 µl of 1M sodium acetate, 700 µl of distilled water and 375 µl of 96% ethanol. Samples prepared on this way were homogenized and incubated at room temperature for 20 minutes. After incubation, the spectrophotometric readings were carried out at a wavelength of 415 nm, compared to the blank. The total flavonoid content in the methanol extracts were calculated with the help of a standard calibration curve (y = 0,1891x; R^2 = 0.9711), and results are reported as catechin equivalents (mg CE/gDW). All measurements were performed in three replications.

Data analysis

The results were statistically analysed using Microsoft Excel 2010, and by software Statistica 8.0 (StatSoft Copyright, Inc. 1984-2007). The calculation of the standard statistical parameters: arithmetic means, standard deviation, the minimum and maximum value was performed for all treatments and control. The statistical evaluation was performed through the analysis of variance (ANOVA) with the Newman-Keuls Post hoc test at the level of statistical significance of 1% ($p < 0.01$).

Investigation of correlations between groups of secondary metabolites was performed using Pearson correlation coefficient. Correlations between the parameters have been analysed at the level of 5%.

RESULTS AND DISCUSSION

Analysis of the total phenolic and flavonoid content

The total phenolic contents in St. John's wort plant extracts using the Folin-Ciocalteu's reagent was expressed in terms of gallic acid equivalent. Results of the Newman-Keuls test indicated a different degree of variation of total phenols in flower methanol extracts of St. John's wort (Figure 2). The maximum content of total phenols in extracts of flowers was recorded for Trebević population, with a mean value of 26.72 mg GAE/gDW, which is statistically significantly higher from the content of total phenolic content measured in flowers of St. John's wort from Dejčići, Majevica, Orjen, Vrelo Bune, Bradina, Novi Šeher and Martin Brod. The minimum content of total phenols was extracted from population of Dejčići (10.55 mg GAE/gDW), and this value was significantly lower than content of phenols in flowers of St. John's wort originating from Velež, Trebević, Poljine, Podveležje, Štrbac, Breka, Hutovo Blato.

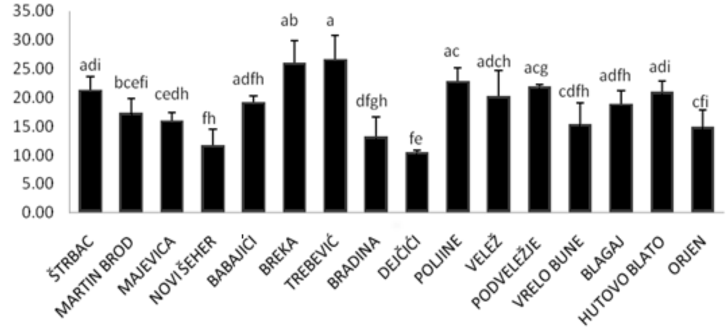


Figure 2. The content of phenols (mg GAE/gDW) in flowers of *H. perforatum* originating from different localities. The phenolic contents in the flower of *H.perforatum* for sites that do not share the same letter are statistically different at the level of 1% ($p < 0.01$) after the analysis of Newman-Keuls post hoc test

The maximum value of the phenol content in extracts of leaves was found in the population of Trebević (24.07 mg GAE/gDW), and statistically significantly higher than phenol content in leaves of populations from localities Dejčići, Majevica, Velež, Orjen, Podveležje, Blagaj, Vrelo Bune, Breka, Bradina, Hutovo Blato, Novi Šeher and Martin Brod (Figure 3). Variation of the secondary metabolites in plant species is a result of the impact of the different abiotic factors such as altitude, geological characteristics of the substrate, etc. (Jakovljević *et al.*, 2013). The combination of environmental factors such as temperature, humidity, precipitation, can speed up or slow down the accumulation of phenolic components. Each type of plant can enter various and specific metabolic processes, and the result is a synthesis of various molecules. Plants which were exposed to low temperatures were typically exposed to oxidative stress showing a reduction in growth, probably due to decreased photosynthesis (Nacif de Abreu & Mazzafero, 2005). It was previously noticed that different abiotic factors such as altitude (Rahnavard *et al.*, 2012; Camas *et al.*, 2014; Cirak *et al.*, 2014), water stress and temperature (Nacif de Abreu & Mazzafero, 2005), salinity (Ramakrishna & Ravishankar, 2011) and combination of various factors in association with genetics and physiological state of the plant (Asadian *et al.*, 2011) influenced the production of secondary metabolites in *Hypericum* species. Phenolic compounds are associated with plant defence mechanisms against various abiotic factors such as water stress, temperature, a high value of UV-B radiation. But it is difficult to isolate one environmental factor and then study his role in production of plant metabolites *in vivo*. Our findings partially confirm the thesis about the existence of a positive correlation between the yield of phenolic components and an increase of altitude. The largest value of total phenol content was noted for localities with altitude from 660 to 1180 meters above the sea level, but, some of the lowest values were also measured in populations harvested at the highest altitudes (Dejčići, Orjen) indicating other factors included in this process. It is very difficult to talk about dominant factor and his greatest impact on the reduction or increase of the production of secondary metabolites, what was indicated in previous studies.

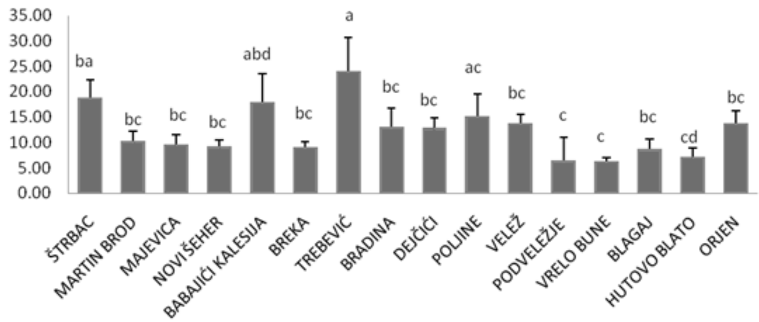


Figure 3. The phenol content (mg GAE/gDW) in leaves of *H. perforatum* originating from different localities. The phenolic contents in the leaves of *H.perforatum* for sites that do not share the same letter, are statistically different at the level of 1% (p<0,01), after the analysis of Newman-Keuls post hoc test

Results of Newman-Keuls analysis of flavonoids, which was performed according to the modified method Ordonez *et al.* (2006), showed that there was no statistical significance in differences between the flavonoids contents in methanol extracts of flowers (Figure 4) of St. John's wort originating from various localities in B&H.

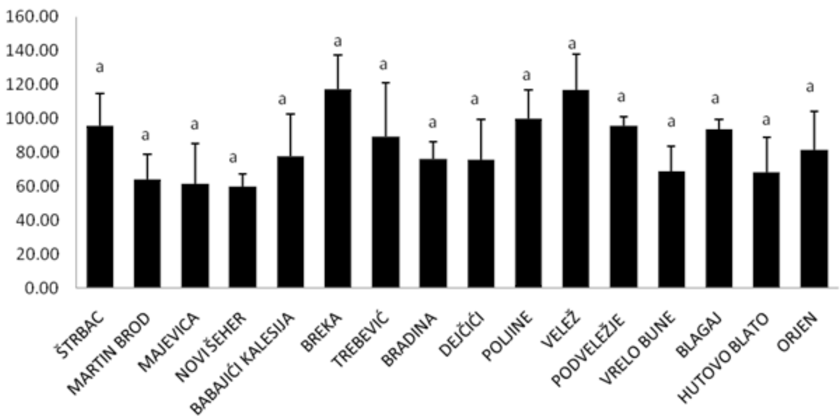


Figure 4. The flavonoid content (mg CE/gDW) in flowers of *H. perforatum* from different localities. The flavonoid contents in the flowers of *H. perforatum* for sites that do not share the same letter, are statistically different at the level of 1% (p<0,01), after the analysis of Newman-Keuls post hoc test

The highest value of the flavonoid content (83.70 mg CE/gDW), in methanol extracts of leaves was determined for locality Štrbac. This value was significantly higher from flavonoid values determined in populations of following localities: Podveležje (21,30 mg CE/gDW), Vrelo Bune (33,71 mg CE/gDW), Hutovo Blato (28,46 mg CE/gDW) and Novi Šeher (35,89 CE mg/gDW) (Figure 5). The minimum value of flavonoid content in leaves was determined for locality Podveležje (21,30 mg CE/gDW). This value was statistically lower from values in populations of localities: Trebević (71,34 mg CE/gDW), Dejčići (69,15 mg CE/gDW), Poljine (68,40 mg CE/gDW), Bradina (62,28 mg CE/gDW)) and Babajići (73,68 mg CE/gDW), as well as from the maximum value, which is determined for locality Štrbac. The amount of flavonoids in population of Hutovo Blato (28,46 mg CE/gDW), was also statistically significantly different from contents of flavonoids in leaves of populations from Štrbac, Trebević, Dejčići, Poljine, and Babajići. The flavonoids content values reported for Majevica, Velež, Orjen, Blagaj, Breka and Martin Brod don’t show statistically significant differences among themselves or in comparison with other localities. These results suggest that plants react in a specific way when they have to face more different stressors at the same time. Plant response cannot be predicted and based on its reaction to isolated stressor what was also previously reported (Germ *et al.*, 2010; Ramakrishna & Ravishankar, 2011; Rahnavard *et al.* 2012; Krasteva *et al.* 2013).

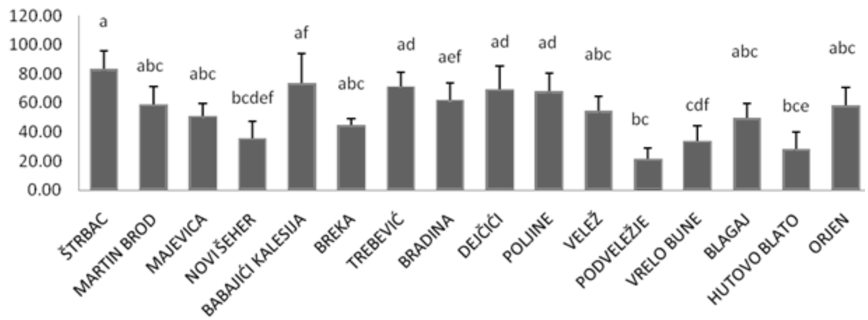


Figure 5. The flavonoid content (mg CE/gDW) in leaves of *H. perforatum*, originating from different localities. The content of flavonoids in the leaves of *H. perforatum* for sites that do not share the same letter are statistically different at level of 1% ($p < 0.01$) after the analysis of Newman-Keuls post hoc test

Comparative analysis of total phenolic and flavonoids content dependent on the plant organ

Total phenolic content of the methanol extracts of Bosnian and Herzegovinian *H. perforatum* varied in different parts of the plant (Figure 6). Statistically significant differences were found between the quantities of phenol in the leaves (6.46 mg GAE/gDW) and flowers (21.89 mg GAE/gDW) of population from Podveležje. Much greater amount of phenol is recorded for the flower (26.06 mg GAE/gDW) than leaves (9.18 mg GAE/gDW) of population from the locality Breka, as well as in the population of Hutovo Blato (leaves 7.19 mg GAE/gDW, flowers 21.07 mg GAE/gDW). Generally, a higher content of phenolic components is presented in flower samples of St. John's wort, regardless of their localities.

Comparative analysis of total flavonoid content in the flowers and leaves (Figure 7), indicated a statistically significant difference, much higher amount of flavonoids in the flowers than in leaves of populations from Velež, Podveležje and Breka. The content of flavonoids in leaves of population from Velež was 54.38 mg CE/gDW, while the content in flowers was significantly higher and amounted to 116.62 mg CE/gDW.

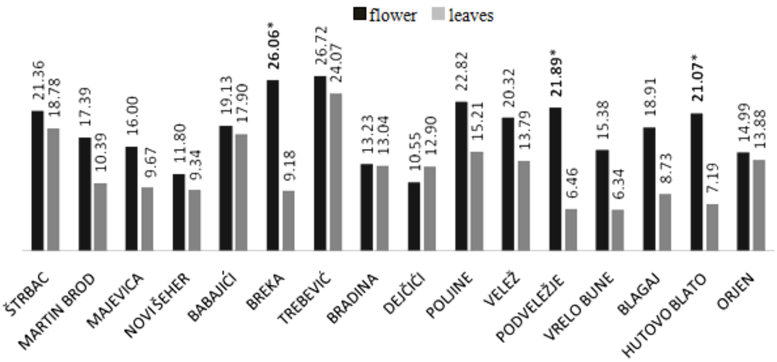


Figure 6. Comparative analysis of phenolic contents (mg GAE/gDW) in flowers and leaves of *H. perforatum* originating from different localities
* significantly higher concentrations of phenols in *H. perforatum* depending on the part of the plant, at the level of 1% (p <0.01) after post hoc Newman-Keuls analysis test

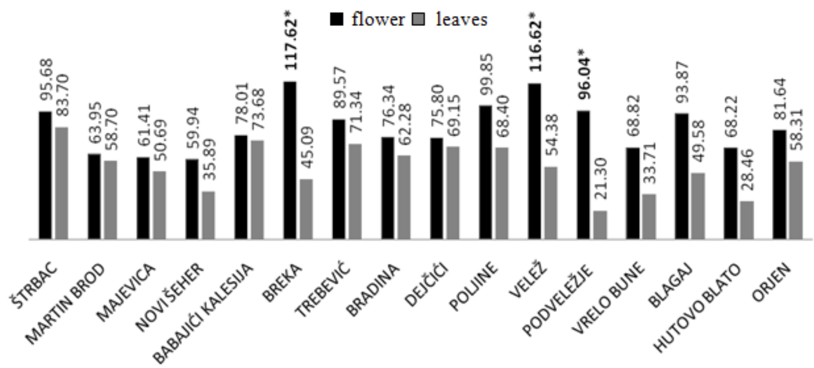


Figure 7. Comparative analysis of flavonoids contents (mg CE/gDW) in flowers and leaves of *H.perforatum*, originating from different localities.
* significantly higher concentrations of flavonoids in *H.perforatum* depending on the part of the plant, at the level of 1% (p <0.01) after post hoc Newman-Keuls analysis test

The content of flavonoids in leaves of population from Podveležje (21,30 mg CE/gDW) was statistically different from the amount of flavonoids in flowers of the same population (96,04 mg CE/gDW). Also, the value of flavonoids in the leaves of population from Breka (45.09 mg CE/gDW) was statistically lower than the amount of flavonoids extracted from the flower of the same population (117.62 mg CE/gDW).
Kazlauskas & Bagdonaite (2006) in their study about bioactive components of *Hypericum maculatum* concluded that the production of secondary metabolites depends largely on phenological phase of the plant. The bioactive compounds accumulate in different types of plant tissues. The concentration of hypericin and

quercetin was higher in flowers and the highest in the flowering stage. The concentration of isoquercitrin and hyperoside accumulated mainly in the leaves during vegetative and budding phases. Camas *et al*. (2012) found that the concentration of the phenolic components depends on the type of plant tissue, therefore the quercitrin, quercetin and mainly isoquercetin were accumulated in the flower of *Hypericum leptophyllum*. The concentration of chlorogenic acid and hyperoside are higher in leaves of *Hypericum leptophyllum*. By comparing the results of research Camas *et al*. (2012) and Kazlauskas & Bagdonaite (2004) it was found that *Hypericum perforatum* accumulate greater amounts of quercetin and quercitrin in flowers and the greater amount of hyperoside in leaves than *Hypericum leptophyllum*. Results in a study about the influence of phenological stages on production of active metabolites in *Hypericum origanifolium* showed increased concentrations of hypericin and quercitrin during full blooming phase, and the highest amount of quercetin is measured during the budding phase (Cirak *et al*., 2007).

The calculated value of Pearsonˈs correlation coefficient (PCC) is $r = 0.415$, at the level of 5% of significance. PCC indicates the positive correlation between phenols and flavonoids, therefore with an increase of phenol content, content of flavonoids also increases in leaves and flowers of St. John's wort populations from different localities in Bosnia and Herzegovina.

CONCLUSION

Results of our study suggest that there were probably different micro-environmental factors which have played a crucial role in the production of plant metabolites, and also, plants can gain other adaptive properties to resolve stress, in addition to increased production of metabolites. Environmental factors that come from outer environment and form the living conditions, precisely biotic and abiotic factors, have a very important influence on production of secondary metabolites. Apart from abiotic factors (light, temperature, water, humidity, air, wind, altitude, exposure, and terrain slope, physical and chemical characteristics of soil) and biotic factors (influences of microorganisms, influence of animals and plants on the plants, anthropogenic influence), production of secondary metabolites may depend on the particular plant organ which we use for extraction, phenological phases of the plant, methods of storage and drying, solvent type, etc.

REFERENCES

Asadian, G., Rahnavard, A., Pourshamsian, K., Ghorbanpour, M. & Taghavi, M. (2011): Study of Variation of Biochemical Components in *Hypericum perforatum* L. Grown in North of IRAN. *Journal of Research in Agricultural Science,* 7(1): 27-36.

Bennett, R. N. & Wallsgrove, R. M. (1994): Tansley Rewiew No. 72 Secondary metabolites in plant defence mechanisms. *New Phytologist,* 127: 617-633.

Camas, N., Radusiene, J., Ivanauskas, L., Jakstas., V. & Cirak, C. (2014): Altidunal changes in the content of bioactive substances in *Hypericum orientale* and *Hypericum pallens*. *Acta Physiologiae Plantarum,* 36: 675-686.

Camas, N., Radusiene, J., Stanius, Z. & Cirak, C. (2012): Secondary Metabolites of *Hypericum leptophyllum* Hochst., an Endemic Turkish Species. *The Scientific World Journal*, (1):501027.

Cirak, C., Radusiene, J., Ivanauskas, L. & Janulis, V. (2007): Variation of bioactive secondary metabolites in *Hypericum origanifolium* during its phenological cycle. *Acta Physiologiae Plantarum*, 29: 197–203.

Cirak, C., Radusiene, J., Ivanauskas, L., Jakstas, V. & Çamas, N. (2014): Changes in the content of bioactive substances among *Hypericum montbretii* populations from Turkey. *Revista Brasileira de Farmacognosia*, 24: 20-24.

Germ, M., Stibilj, V., Kreft, S., Gaberščik, A. & Kreft, I. (2010): Flavonoid, tannin and hypericin concentrations in the leaves of St. John´s wort (*Hypericum perforatum* L.) are affected by UV-B radiation levels. *Food Chemistry,* 122: 471-474.

Glišić, S. B., Popadić, S. V. & Skala, D. U. (2006): KANTARION (*Hypericum perforatum* L.) –Natkritična ekstrakcija, antimikrobna i antidepresivna aktivnost ekstrakta i pojedinih komponenti. *Hemijska Industrija*, 60(3-4): 61-71.

Jakovljević, Z. D., Stanković, S. M. & Topuzović, D. M. (2013): Seasonal variability of *Chelidonium majus* L. secondary metabolites content and antioxidant activity. *EXCLI Journal*, 12: 260-268.

Kazlauskas, S. & Bagdonaite, E. (2004): Quantitative analysis of active substances in St. John's wort (*Hypericum perforatum* L.) by the high performance liquid chromatography method. *Medicina (Kaunas*), 40(10): 975-981.

Kazlauskas, S. & Bagdonaite, E. (2006): Secondary metabolites variation in *Hypericum maculatum. Acta Biologica Universitatis Daugavpiliensis,* 6(1-2): 39-44.

Krasteva, I. Nedelcheva, A., Pavlova, D., Zdraveva, P., Nikolov, S. & Mitov, K. (2013): Influence of serpentine soils on the flavonoid content of *Hypericum* populations growing in Bulgaria. *African Journal of Pharmacy and Pharmacology*, 7(25): 1762-1765.

Maleš, Ž., Brantner, A. H., Sović, K. & Hazler-Pilepić, K. (2006**):** Comparative phytochemical and antimicrobial investigations of *H. perforatum* L. subsp. *perforatum* and *H. perforatum* L. subsp. *angustifolium* (DC.) Gaudin. *Acta Pharmaceutica*, 56: 359–367.

Nacif de Abreu, I. & Mazzafera, P. (2005): Effect of water and temperature stress on the content of active constituents of *Hypericum brasiliense* Choisy. *Plant Physiology and Biochemistry*, 43: 241-248.

Odabas, S. M., Radugienë, J., Camas, N., Janulis, V., Ivanauskas, L. & Çırak, C. (2009): The quantitative effects of temperature and light intensity on hyperforin and hypericins accumulation in *Hypericum perforatum* L. *Journal of Medicinal Plants Research*, 3(7): 519-525.

Ordonez, A., Gomez, J., Vattuone, M. & Isla, M. (2006**):** Antioxidant activities of *Sechium edule* (Jacq.) Swart extracts. *Food Chemistry*, 97: 452-458.

Pandey, K. B. & Rizvi, S. I. (2009): Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity*, 2(5): 270-278.

Pirbalouti, A. G., Rahnama, G. H., Malekpoor, F. & Broujeni, H. R. (2011): Variation in antibacterial activity and phenolic content of *Hypericum scabrum* L. populations. *Journal of Medicinal Plants Research*, 5(17): 4119-4125.

Rahnavard, A., Daneshian, J., Heravan, E. M., Valadabadi, S. A. & Golein, B. (2012): Investigation of the most important secondary metabolites of St. John's wort *(Hypericum perforatum* L.) in Caspian climate. *Journal of Food, Agriculture and Environment*, 10(2): 375-381.

Ramakrishna, A. & Ravishankar, G. A. (2011**):** Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling and Behavior*, 6(11): 1720-1731.

Walter, M. & Marchesan, E. (2011): Phenolic Compounds and Antioxidant Activity of Rice. *Brazilian Archives of Biology and Technology*, 54: 371-377.

Wolfe, K., Wu, X. & Liu, R. (2003): Antioxidant activity of apple peels. *Journal of Agricultural and Food Chemistry*, 51: 609-614.

EVALUATING MEMBER SATISFACTION IN AGRICULTURAL COOPERATIVES: A CASE OF DAIRY COOPERATIVES IN IZMIR PROVINCE*

Filiz Kinikli[1], Murat Yercan[1]

Original scientific paper

Summary

This study attempts to assess member satisfaction in agricultural cooperatives by use of SERVQUAL model. The SERVQUAL model was developed in 1988 by Zeithaml, Parasuraman and Berry as a multi-item scale developed to assess customer perceptions of service quality in service industry. Cooperatives have a dual nature of specification which members are the costumers in all cooperative activities. Customers/members judge service quality as low or high depending upon whether the service performance meets their expectation or not. The research is based on the survey study. The population of research was the dairy cooperative members in İzmir Province in Turkey (N=5731). So, sample of this study has formed from 116 members. Using SERVQUAL tool, five service quality dimensions using two segments in the form of a questionnaire consisting of 22 questions each have been used for the customers/members. Data were analyzed by SPSS 20 using descriptive statistics and by using data, perceived and expected service quality was evaluated comparatively with gap analysis. Besides, customers'/members' dimensions of perceived service quality were investigated and evaluated by statistical tests for demographic differences. According to questionnaire results, the expected service quality was found greater than the perceived service quality. The general service quality perceived by the members of cooperatives which process the raw material was found greater than the members of cooperatives which collect the the raw material.

Key words: *Dairy, cooperatives, satisfaction, service quality*

INTRODUCTION

Due to nature, agriculture is exposed to various risks during the period from production to marketing. Farmers organization is a way of reducing the risks in agriculture. Agricultural co-operatives form the basis of farmers organization (Yercan, 2003).

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Ege University, Faculty of Agriculture, Department of Agricultural Economics, Turkey

Cooperatives are social-economic organizations which contribute economic, social, and environmental sustainability. Cooperatives are different from country to country according to the working principle. But, in general, they serve the same purposes. Cooperatives are a major part of the global economy.
The satisfaction of cooperative members is very important for the development of cooperatives. If cooperatives did not meet the expectations of the cooperative members (such as price, market, income, employment, risturn etc.), they may be failure.
In the cooperatives success and performance, the role of cooperative relations with its members is very important. In this respect, various studies have been done in the literature for many years about cooperative-members relations (Kubaş, 1992; Özdemir, 1996; Ertan ve Turan, 2001; Eken, 2010; Kılıç, 2011; Ertan ve Kaya, 2012). In the majority of the studies done, it is stated that the cooperatives fail because of the lack of trust and the lack of expectations.

When the other studies conducted in the world are examined, it is seen that the "human" factor is important in the success of the cooperatives and the studies which the attitudes and behaviors of the members are analyzed have become widespread (Bhuyan, 2007; Xiang and Sumelius, 2010).
As a result of the work carried out in the world and in Turkey, it is emphasized that the cooperative members should cooperate with the cooperatives for success of the cooperatives. Unfortunately, the cooperative members are low the level of cooperate with their cooperatives in Turkey. The reason is that, cooperative members' expectation are not supplied with their cooperatives. Therefore, cooperatives' service quality is very important for cooperatives success. The scientific studies are very few about cooperatives service quality in the world (Wilson *et al*., 2011; Ebrahimi and Imani, 2014; Balina, 2015; Sharma, 2016). In Turkey, no study of the cooperative members' perception of service quality was found.
The main purpose of this study is to examine member satisfaction in agricultural cooperatives by use of SERVQUAL model in dairy cooperatives in İzmir. The objectives of our study can categorize in 4 groups. The first socio-economic characteristics of dairy farmers, secondly their level of cooperation with cooperatives, thirdly their level of perceived and expected service quality from cooperatives and finally which cooperative has got high service quality is examined.

MATERIALS AND METHODS

The main material of this study constituted from dairy farmers who are member of dairy cooperatives in İzmir province. Data are gathered from face to face interview method by using survey form and also some proceedings, articles, reports and thesis which are related to the subject, has been used as a secondary data sources. Surveys have applied only dairy farmers who are member of dairy cooperatives in İzmir province.

The total number of Dairy Cooperatives which process the raw material and collect the the raw material that actively work in the province of İzmir is 15. The number of cooperatives which process the raw material is 4 and the number of cooperatives which collect the the raw material is 11 (Table 1).

Table 1: Cooperatives within the scope of the research and number of members

Cooperatives within the scope of the research *		**Number of Members**
Cooperatives which process the raw material	Bağarası-Yenibağarası Agricultural Development Cooperatives	170
	İğdeli Agricultural Development Cooperatives	1841
	Tire Agricultural Development Cooperatives	1279
	Bademli Fidancılık Agricultural Development Cooperatives	237
	TOTAL	**3527**
Cooperatives which collect the the raw material	Süt Üreticileri Agricultural Development Cooperatives	56
	Aşağıcuma Köyü Agricultural Development Cooperatives	120
	Gerenköy Agricultural Development Cooperatives	80
	Haliller Agricultural Development Cooperatives	316
	Aydoğdu Agricultural Development Cooperatives	74
	Adagüme Agricultural Development Cooperatives	663
	Çaylı Agricultural Development Cooperatives	176
	Kaymakçı Agricultural Development Cooperatives	235
	Kayaköy Agricultural Development Cooperatives	144
	Gereli Agricultural Development Cooperatives	172
	Ovakent Agricultural Development Cooperatives	168
	TOTAL	**2204**

Source: *İzmir provincial directorate of agriculture

Proportional sampling method formula which is shown below is used to calculate sampling size (Newbold, 1995).

$$n = \frac{Np(1-p)}{(N-1)\sigma_{px}^2 + p(1-p)}$$

n: sample size
N: Number of members in Agricultural Development Cooperatives
p: the percentage of members (takes as 0.5 to reach maximum sample size)
σ_{px}^2: variance.

The sample was chosen by the formula and probability was taken 0.50 to get the posible largest sample size. According to the proportional sampling method, with a

95% confidence interval and 9% margin of error, the required sample size was found to be 116. Sample size was proportionately distributed among the cooperatives.

SERVQUAL tool which developed by Parasuraman *et al.* in 1985 was used to measure the opinions of cooperative members' about the cooperatives on service quality in survey.

The SERVQUAL scale is a survey instrument which claims to measure the service quality in any type of service organization on five dimensions which are tangibles, reliability, assurance, responsiveness and empathy (Parasuraman *et al.*, 1988).

Measuring service quality has been one of the most recurrent topics in management literature, Parasuraman *et al.*, (1988), Gronroos, (1984), Cronin *et al.*, (1992).

This model measures service quality using five distinct dimensions that can be considered as indicators of construct of perceived service quality. The five dimensions of SERQUAL are "Tangibles", "Reliability", "Responsiveness", "Assurance", and "Empathy" as described in Table 2.

Table 2: Five distinct dimensions in SERQUAL model

Dimension	**Description**
Tangibles	The appearance of physical facilities, equipment, personnel and communication materials
Reliability	The ability to perform the promised service dependably and accurately
Responsiveness	The willingness to help customers and provide prompt service
Assurance	The knowledge and courtesy of employees and their ability to convey trust and confidence
Empathy	The caring, individualized attention the firm provides its customers

Source: Ebrahimi and Imani, 2014

The SERVQUAL model was adopted to cooperatives by doing little change. Using SERVQUAL tool, five service quality dimensions using two sections in the form of a questionnaire consisting of 22 questions each have been used for the customers/members. Statements in both sections used a seven-point Likert scale ranging from ‘‘Strongly Agree’’ (7) to ‘‘Strongly Disagree’’ (1).

Relations between expected service and perceived service are expressed as follows:

Expected Service > Perceived Service; service quality is low

Expected Service = Perceived Service; satisfactory service quality

Expected Service < Perceived Service; service quality is high

Data were analyzed by SPSS 20 using descriptive statistics and by using data, perceived and expected service quality was evaluated comparatively with gap analysis. Reliability analysis was carried out to check out whether the data sets were reliable. The normal distribution test was done with the Kolmogorov-Smirnov test for continuous variables. Variance analysis was applied for the normal distribution variables. It was determined that the variables did not show normal distribution. Mann-Whitney U test was applied for the variables.

RESULTS AND DISCUSSION

General Characteristics of survey participants

All participants were male, averagely 47 years old and graduated from primary school. One-way ANOVA test was used to determine whether there was a statistically significant difference between groups for age. The difference between the groups was found to be significant for $p \leq 0.10$.

The average household size is about 5 people which is higher than İzmir Province average (3.04 people TURKSTAT) in 2016. It is expected that members' household size should be above the average of Izmir because of the rural life. 92,2% of the members are married and 7,8% of the members are single.

The members'general farming experience is 24,35 year and the animal husbandry experience is 22,00 year. The members of cooperatives which process the raw material animal husbandry experience and farming experience are longer period of time than members of cooperatives which collect the raw material. Mann-Whitney U test was used to determine whether there was a statistically significant difference between groups for farming experience and animal husbandry experience. The difference between the groups was found to be significant for $p \leq 0.05$. 75,9% of the members has not non-agricultural income. 24,1% of the members has non-agricultural income. The average non-agricultural income is 450,52 €. Total Monthly income is 2072,93 € (Table 3).

Table 3: Demographic characteristics of the participants surveyed

		Survey Participants (%) or Average		
		Cooperatives which collect the raw material (N:45)	Cooperatives which process the raw material (N:71)	General (N:116)
Age*		45,00	48,62	47,22
Education level (year)**		7,62	6,24	6,78
Household size (people)		4,58	4,82	4,72
Marital status	Married	91,1%	93,0%	92,2%
	Single	8,9%	7,0%	7,8%
Farming Experience (Year) **		20,16	26,99	24,34
Animal Husbandry Experience (Year) **		18,31	24,34	22,00
Non-agricultural income	Yes	17,8%	28,2%	24,1%
	No	82,2%	71,8%	75,9%
Non-agricultural income (Euro)		480,18	438,85	450,52
Total Monthly income (Euro)		2355,45	1893,50	2072,93

*According to One-Way Anova test, the difference between the groups was significant for $p \leq 0.10$.

**According to Mann-Whitney U test, the difference between the groups was found to be significant for $p \leq 0.05$.

Characteristics of the farms in the research area

Characteristics of the farms in the research area is examined that members have 14,58 dairy cow. The members of cooperatives which collect the raw material dairy cow number is more than the members of cooperatives which process the raw material. There was a statistically significant different between the groups ($p \leq 0.05$). Daily milk yield data is examined that the members of cooperatives which collect the raw material daily milk yield data is more than the members of cooperatives which process the raw material. This situation is directly proportional to the dairy cow number. There was a statistically significant different between the groups ($p \leq 0.05$). The members of cooperatives which collect the raw material sell about 278,22 liter milk to the cooperatives every day. The members of cooperatives which process the raw material sell about 227,96 liter milk to the cooperatives every day. This number is lower than the other. Because the members of cooperatives which process the raw material have less dairy cow than the members of cooperatives which collect the raw material. Cooperatives paid to members for raw milk approximately 0,265 euro. If members sell milk to merchants, they paid to members for raw milk appeoximately 0,258 euro. This price is lower than cooperatives' price (Table 4).

Table 4: Characteristics of the farms in the research area

	Survey Participants (%) or Average		
	Cooperatives which collect the raw material (N:45)	Cooperatives which process the raw material (N:71)	General (N:116)
Dairy Cow **	18,93	11,82	14,58
Daily Milk Yield Data(lt)**	333,98	228,08	269,16
Daily milk amount given to cooperatives (lt)	278,22	227,96	247,46
Raw milk price paid to members by cooperatives (Euro)	0,266	0,263	0,265
Raw milk price paid to members by merchants (Euro)	0,253	0,262	0,258

**According to Mann-Whitney U test, the difference between the groups was found to be significant for $p \leq 0.05$.

The cooperative members' milking style is examined that 90,5 % of the members use milking machine. 58,6 % of the members use steel container, 31,0% of the members use refrigerated tank for storing the milk. The majority of the members of cooperatives which collect the raw material transport milk themselves or are transported from their home. 73,2 % of the members of cooperatives which process the raw material are transported their milk by village collector. 90,5 % of the cooperatives pay to their members on monthly. Milking style, milk storage and payment term are examined that there was a statistically significant different between the groups ($p \leq 0.05$) (Table 5).

Table 5: Characteristics of the farms in the research area

		Survey Participants (%)		
		Cooperatives which collect the raw material (N:45)	Cooperatives which process the raw material (N:71)	General (N:116)
Milking style**	Hand	2,2	14,1	9,5
	Milking Machine	97,8	85,6	90,5
Milk storage**	Plastic container	2,2	15,5	10,3
	Steel container	53,3	62,0	58,6
	Refrigerated Tank	44,4	22,5	31,0
Collection of milk by cooperatives	From the members' home	47,7	9,9	24,1
	Village collector	0,0	73,2	44,8
	In person	52,3	16,9	30,2
Payment term**	Cash	11,4	0	4,3
	Every 15 days	11,4	0	4,3
	Monthly	77,3	100	90,5

**According to Mann-Whitney U test, the difference between the groups was found to be significant for $p \leq 0.05$.

Service Quality Assessment of the members in the research area

SERVQUAL methodology is proven to be a powerful tool to analyze the member satisfaction in different sections of science and industry. The standard SERVQUAL questionnaire including the two parts of individual characteristics and the five service quality dimensions was used for data collection. These two parts consists of expected service quality and perceived service quality.

The results of research showed that for each expected service dimension based on SERQUAL model, (considering the table 6) expected service quality score is over 6. This score is very high for real service quality. Apart from empathy dimension every expected service dimension and general expected service quality were a statistically significant different between the groups ($p \leq 0.05$).

Table 6: Expected Service Quality

	Survey Participants Average		
	Cooperatives which collect the raw material (N:45)	Cooperatives which process the raw material (N:71)	General (N:116)
Tangibles **	6,84	6,14	6,42
Reliability **	7,00	6,39	6,62
Responsiveness **	6,99	6,51	6,70
Assurance **	6,99	6,74	6,84
Empathy	6,88	6,85	6,86
General Expected Service Quality**	6,94	6,53	6,69

**According to Mann-Whitney U test, the difference between the groups was found to be significant for p≤0.05.

The perceived service quality is examined that every perceived service dimension and general percieved service quality were a statistically significant different between the groups (p≤0.05). The members of cooperatives which collect the raw material perceived service quality is less satisfied the members of cooperatives which process the raw material (Table 7).

Table 7: Perceived Service Quality

	Survey Participants Average		
	Cooperatives which collect the raw material (N:45)	Cooperatives which process the raw material (N:71)	General (N:116)
Tangibles **	4,97	6,69	6,02
Reliability **	5,64	6,77	6,33
Responsiveness **	5,62	6,80	6,34
Assurance **	5,51	6,58	6,17
Empathy **	5,66	6,23	6,01
General Percieved Service Quality**	5,48	6,61	6,17

**According to Mann-Whitney U test, the difference between the groups was found to be significant for p≤0.05.

The table 8 is showed that for each service dimension based on SERVQUAL model, there was gap in every aspect of service quality. There was a quality gap in all service quality dimensions and the related measuring phrases.
The highest average of quality gap was observed in the empathy (-0,85) and then in assurance (-0,67) tangibles (-0,39), and responsiveness (-0,36), respectively. The lowest average belonged to reliability (-0,29).

Table 8: Gap Analysis

	Survey Participants Average		
	Cooperatives which collect the raw material (N:45)	Cooperatives which process the raw material (N:71)	General (N:116)
Tangibles **	-1,88	0,55	-0,39
Reliability **	-1,36	0,38	-0,29
Responsiveness **	-1,37	0,28	-0,36
Assurance **	-1,49	-0,16	-0,67
Empathy **	-1,22	-0,61	-0,85
General Service Quality**	-0,73	0,04	-0,26

**According to Mann-Whitney U test, the difference between the groups was found to be significant for $p \leq 0.05$.

The results of research showed that there was a statistically significant different between expectations and perceptions of services in dairy cooperatives, the gap in different dimensions of services quality was significant ($p<0.05$). In other words there is a significant difference between every aspect of expectation and perception members in all dimensions of services in dairy cooperatives members' viewpoint.

CONCLUSION

The research findings also confirmed that, although the SERVQUAL scale was a very useful tool as a concept, it needed to be adapted for the specific service segments and for the cultural context within which it was used (Akbaba, 2006).

The result of research showed that in general, the service quality of cooperatives which process the raw material is better than the service quality of cooperatives which collect the the raw material. And the expected service quality was found greater than the perceived service quality.

The members of cooperatives which process the raw material expected service quality score is lower than the members of cooperatives which collect the raw material. Whereas, the members of cooperatives which collect the raw material perceived service quality is less satisfied the members of cooperatives which process the raw material.

For each service dimension based on SERVQUAL model, there was gap in every aspect of service quality. The highest average of quality gap was observed in the empathy (-0,85) and the lowest average belonged to reliability (-0,29). The scores for the tangibles, reliability, responsiveness, assurance and empathy reflect significant differences. In other words, they indicated that there were gaps among the observations and expectations of clients in terms of these dimensions.

According to the service quality dimensions, the lowest average of service quality dimension for the members of cooperatives which process the raw material was "Empathy" dimension. This situation is showed that cooperative management wasn't interested with the members. Also, this situation is showed that communication between the cooperative management and the members wasn't sufficient. The lowest average of service quality dimension for the members of cooperatives which collect the raw material was "Tangibles" dimension. This situation is showed that cooperatives which collect the raw material haven't got enough buildings, machinery, technologies etc.. Many cooperatives haven't got an executive office.

Service quality dimensions (tangibles, reliability, responsiveness, assurance and empathy) are so different for the two types of cooperatives. Cooperatives which collect the raw material are very small-scale cooperatives in Turkey. And these cooperatives' tangibles dimension which is consists of physical facilities, equipment, personnel and communication materials is not enough. Whereas, cooperatives which process the raw material are full-scale cooperatives in Turkey. And these cooperatives' tangibles dimension which is consists of physical facilities, equipment, personnel and communication materials is enough. So these cooperatives provide service better than small-scale cooperatives. This situation do not derive from the payment scheme, organization of the milk pick up or other reasons. The only reason why the difference in service quality dimensions (tangibles, reliability, responsiveness, assurance and empathy) between the two types of cooperatives is so different is the scale of the cooperatives.

According to the finding, there is to suggest several actions to reduce the amount of service gaps:

Cooperatives which process the raw material should communicate more frequently with their members. More social activities should be organized with members. Education or recruiting staff that understand the importance of service and have the aptitude to provide the members with effective resolutions on the first contact whenever possible. The measuring and monitoring of customers and members complaints is vital and organization must have suitable systems and commitment to do this.

REFERENCES

Akbaba, A. (2006): Measuring service quality in the hotel industry: A study in a business hotel in Turkey. International Journal of Hospitality Management, Volume 25, Issue 2, Pages 170–192.

Balina, R. (2015): Financial Services Costs in Poland's Cooperative Banking in Opinion of Small and Medium-size Enterprise, Management Studies, February 2015, Vol. 3, No. 1-2, 34-40.

Bhuyan, S. (2007): The People Factor in Cooperatives: An Analysis of Members' Attitudes and Behavior, Canadian Journal of Agricultural Economics, 55, 275-298.

Ebrahimi, M. S., Imani, F. (2014): Evaluating Customer Satisfaction in Iranian Agricultural Cooperatives by use of SERVQUAL Model, International

Journal of Agriculture and Crop Sciences. Available online at www.ijagcs.com IJACS/2014/7-4/202-206 ISSN 2227-670X

Eken, M. (2010): Tüketim Kooperatiflerinde Ortak-Kooperatif İlişkisi: Damla Tüketim Kooperatifi Örneği, Yüksek Lisans Tezi,Gazi Üniversitesi Sosyal Bilimler Enstitüsü, s. 87.

Ertan, A., Kaya, M. S. (2012): Çünür Sulama Kooperatifinde Kooperatif-Ortak İlişkilerinin Analizi, Süleyman Demirel Üniversitesi Sosyal Bilimler Enstitüsü Dergisi Yıl: 2012/2, Sayı:16.

Ertan, A., Turan, A. (2001): Gülbirlik Ortaklarının Kooperatif Ortak İlişkisi Yönünden Analizi, SDÜ İ.İ.B.F. Dergisi, C.6, S.2, s. 29-43.

Gronroos, C. (1984): A Service Quality Model and its Marketing Implications, European Journal of Marketing, Vol.18, No.4, pp.36-44.

Kılıç, B. (2011): Samsun İlindeki Tarımsal Kalkınma Kooperatiflerinde Ortak-Kooperatif İlişkilerinin Analizi, Yüksek Lisans Tezi, Ondokuz Mayıs Üniversitesi Fen Bilimleri Enstitüsü, s.153.

Kubaş, A. (1992): Anamur Pamuk, Yerfıstığı ve Yağlı Tohumlar Tarım Satış Kooperatifinin Ekonomik Yapısı ve Kooperatif Ortak İlişkileri, Yüksek Lisans Tezi, Trakya Üniversitesi Fen Bilimleri Enstitüsü, s. 106.

Özdemir, G. (1996): Tekirdağ İlinde Tarımsal Kooperatiflerde Kooperatif Ortak İlişkileri, Doktora Tezi, Ankara Üniversitesi Fen Bilimleri Enstitüsü, s.233.

Parasuraman, A., Zeithaml, V. and Berry, L. L. (1988): SERVQUAL: MultipleItem Scale for Measuring Consumer Perceptions of Service Quality, Journal of Retailing, 64.1 (Spring).

Sharma, S. (2016): Using SERVQUAL to Assess the Customer Satisfaction Level: A Study of an Urban Cooperative Bank, Journal of Economics and Public Finance ISSN 2377-1038 (Print) ISSN 2377-1046 (Online) Vol. 2, No. 1, 2016.

Wilson, N., Hallb, T., Fieldsc, D. (2011): Measuring Retail Service Quality in Farm Supply Cooperatives, International Food and Agribusiness Management Review Volume 14, Issue 1, 2011.

Xıang, L. Y., Sumelıus, J. (2010): Analysis of the Factors of Farmers' Participation in the Management of Cooperatives in Finland. Journal of Rural Cooperation, 38(2): 134-135.

Yercan, M. (2003): Tarım Kooperatifleri, Ege Üniversitesi Tarımsal Uygulama ve Araştırma Merkezi, Çiftçi Broşürü:34.

EKONOMSKI EFEKTI PRIMENE MEHANIZACIJE U BERBI MALINE*

ECONOMIC EFFECTS OF MECHANIZATION IN HARVESTING RASPBERRY

Todor Marković[1], Željko Kokot[1], Merima Makaš[2]

Originalni naučni rad

Rezime

Zahvaljujući geografskom položaju, klimatskim i zemljišnim uslovima, Srbija ima veoma povoljne uslove za voćarsku proizvodnju. Poseban značaj ova proizvodnja ima u brdsko-planinskom području, gde čini preko 90% ukupnih poljoprivrednih površina. Međutim, jedan od limitirajućih faktora kod voćarske proizvodnje svakako je berba. Pored permanentnog problema nedostatka radne snage, sa kojim se voćari iz godine u godinu sve više susreću, angažovanje postojeće radne snage praćeno je nizom drugih problema, pre svega u pogledu prevoza, smeštaja, ishrane i zdravstvene zaštite. Ovi problemi mogu biti prevaziđeni korišćenjem mehanizacije u berbi, obzirom da savremena tehnologija gajenja, između ostalog, podrazumeva i zamenu manuelnog rada mašinskim. Primenom mehanizovane berbe znatno se skraćuje vremenski period branja, čime se ujedno postiže i bolji kvalitet ubrane maline. Na ovaj način troškovi berbe smanjuju se za čak 78%. Smanjenje troškova berbe značajno se odražava na krajnji finansijski rezulatat, obzirom da ovi troškovi učestvuju i do 65% u strukturi ukupnih troškova proizvodnje maline.

Ključne reči: *finansijski rezultat, branje, mehanizacija, troškovi proizvodnje, malina*

Summary

Due to its geographical position, the climatic and soil conditions, Serbia has very favorable conditions for fruit production. Special significance this production has in highland areas, where makes over 90% of total agricultural area. However, one of the limiting factors in fruit production is definitely harvest. In addition to the permanent problem of lack of human labor, with which fruit growers are facing more and more, from year to year, engagement of the existing human labor is accompanied by a series of other problems, especially in terms of transportation, accommodation, food and health care. These problems can be resolved by using mechanization in harvesting,

* Rad je rezultat istraživanja na projektu Ministarstva obrazovanja, nauke i tehnološkog razvoja Republike Srbije broj III46006, pod nazivom: „Održiva poljoprivreda i ruralni razvoj u funkciji ostvarivanja strateških ciljeva Republike Srbije u okviru Dunavskog regiona". Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Poljoprivredni fakultet, Univerzitet u Novom Sadu, Srbija
[2] 2Poljoprivredno-prehrambeni fakultet, Univerzitet u Sarajevu, B&H

considering that modern breeding technology, among other things, includes the replacement of manual labor by machines. Using the mechanized harvesting of raspberry significantly shortens the period of harvesting, while in the same time the better quality of the harvested raspberry is achieved. Thus the costs of harvesting are reduced by as much as 78%. Reduction of costs of harvesting significantly affects the final financial result, considering that these costs participate up to 65% in total raspberry production costs.

Key words: *financial result, harvesting, mechanization, production costs, raspberry*

UVOD

Na osnovu svog geografskog položaja, kao i klimatskih i zemljišnih uslova, Srbija ima vrlo povoljne uslove za voćarsku proizvodnju. Ona dominira u brdsko-planinskom području, gde čini preko 90% ukupnih poljoprivrednih površina. Ipak, berba predstavlja jedan od limitirajućih faktora daljeg razvoja voćarske proizvodnje. Osnovni problem čini nedostatak radne snage, sa kojim se voćari iz godine u godinu susreću. Angažovanje postojeće radne snage prati niz problema, pre svega prevoz, smeštaj, ishrana i zdravstvena zaštita. Korišćenjem mehanizacije u berbi ovi problemi mogu biti prevaziđeni, budući da savremena tehnologija gajenja podrazumeva između ostalog i zamenu ručnog rada mašinskim. Primenom mehanizacije ne samo da se smanjuju troškovi proizvodnje, već se i trajanje berbe znatno skraćuje, što se pozitivno odražava na kvalitet ubranog voća.

Za ubiranje voća karakteristično je, osim angažovanja velikog broja radnika, dugog vremenskog perioda i velikog udela troškova koji se odnose na manipulaciju sa ubranim plodovima, i visok udeo transportnih troškova. Pojavom mehanizovanog ubiranja znatno se skratio vremenski period branja po stablu, a samim tim i ukupni troškovi berbe voća. Mogućnost primene mehanizacije, pre svega, odnosi se na velike plantaže zasada, prvenstveno špalirne, mada je znatna ekonomska opravdanost postignuta i pri branju pojedinačnih stabala, kao što su orah i maslina (Živković i Veljić, 2011). Gajenje voća na velikim površinama, kao i visoki prinosi koji se ostvaruju, ukazuju, ne samo na opravdanost, već i na potrebu korišćenja tehničkih rešenja za mašinsko branje, najčešće putem otresanja stabala. Preduslovi za ovakav pristup branja su usklađivanje zasada i tehnologije gajenja mašinama, a sa druge strane, usklađivanje tehničkih sistema, kroz optimizaciju brojnih tehničkih parametara, stablu, žbunu, pa i plodu (Veljić i sar., 2009).

Kao primer, može se uzeti činjenica da troškovi ručne berbe čine i do 70% ukupnih troškova proizvodnje maline. Taj podatak upućuje na nužnost mehanizovanja procesa berbe, kako bi se u odnosu na ručno branje skratilo vreme izvođenja operacije, smanjio broj radnika, povećala proizvodnost i smanjili troškovi (Urošević i sar., 2011). Sa druge strane, ne sme se zanemariti ni činjenica da je kvalitet mašinski ubranih plodova malo lošiji u odnosu na ručno ubrane, ali su ipak plodovi pogodni za preradu (Mika *et al.*, 2012).

Cilj istraživanja u ovom radu jeste analiza ekonomske opravdanosti primene mehanizacije, konkretno samohodnog kombajna, u berbi maline. Ova voćna vrsta nalazi se u fokusu budući da je Srbija, uz Rusiju i Poljsku, najveći proizvođač u Evropi (Veljković Biljana i sar., 2006). Činjenica da se poljoprivreda Srbije susreće sa stalnim nedostatkom sezonske radne snage, posebno u periodu berbe maline, još više daje na značaju ovoj problematici.

MATERIJAL I METOD RADA

Za potrebe realizacije postavljenog cilja istraživanja korišćeni su podaci individualnog poljoprivrednog gazdinstva, iz Kruščića (AP Vojvodina, Srbija), koje se bavi uzgojem maline, zatim podaci Republičkog zavoda za statistiku i kompanije SZR "Elektronik" čija je delatnost proizvodnja specijalizovanih kombajna za berbu maline.

U radu je analizirana mogućnost primene mehanizacije u berbi maline. Osim analize prednosti mehanizovane berbe u odnosu na ručnu, utvrđeni su i ukupni eksploatacioni troškovi, kao i najvažniji pokazatelji ekonomske efikasnosti primene mehanizovane berbe.

Produktivnost je pokazatelj efikasnosti živog rada, koji predstavlja odnos između količine proizvoda i ljudskog rada koji je utrošen za njegovu proizvodnju. Izračunava se po opštoj formuli:

$$P_r = \frac{Q}{L} \tag{1}$$

gde je:
P_r – produktivnost rada
Q – obim proizvodnje
L – uloženi (ljudski) rad

Ekonomičnost je takođe pokazatelj efikasnosti i predstavlja odnos između ostvarene vrednosti prizvodnje i utrošenih činilaca proizvodnog procesa (rada, sredstava za rad i predmeta rada). Stepen ekonomičnosti se iskazuje koeficijentom ekonomičnosti i izračunava se primenom sledećeg obrasca:

$$E = \frac{VP}{UT} \tag{2}$$

gde je:
E – ekonomičnost
VP – vrednost proizvodnje
UT – ukupni troškovi berbe

Rentabilnost predstavlja jedan od osnovnih principa ekonomije i jedan od pokazatelja finansijskog uspeha. Izražava se zahtevom da se određenim ulaganjem sredstava (kapitala) ostvari što veći finansijski rezultat, odnosno da se određeni finansijski rezultat ostvari sa što manjim ulaganjem. U suštini predstavlja odnos između ostvarenog finansijskog rezultata i uloženih osnovnih i obrtnih sredstava. Stopa rentabilnosti, ili kako se još naziva stopa dobiti proizvodnje, utvrđuje se prema obrascu:

$$R = \frac{D_b}{VP} \cdot 100 \qquad (3)$$

gde je:
R – stopa rentabilnosti
D_b – dobit

REZULTATI ISTRAŽIVANJA

Kombajn za branje jagodičastog voća (konkretno maline i kupine) konstruisan je kao samohodna poljoprivredna mašina, koja vrši branje pomoću izazivanja vibracija u krošnji voćke, što dovodi do opadanja plodova. Ovakvim vidom berbe omogućava se efikasno opadanje samo tehnološki zrelih plodova bez oštećenja. Kombajn poseduje specijalno razvijeni sistem automatske nivelacije koji omogućava upotrebu berača i na terenima pod nagibom, odnosno na plantažama koje se nalaze na veoma nepristupačnim mestima u našoj zemlji. Reč je o samohodnoj mašini sa hidrauličnim pogonom na svim točkovima. Težina berača je 3.000 kg, širina 2,7 m, visina 2,9 m i dužina 4,7 m. Za neometan rad potrebna su svega tri radnika koja će opsluživati mašinu (Slika 1).
Posmatrajući način rada, u prednjem delu kombajna, sa obe strane su vertikalno postavljene „četke“ sa plastičnim prstima. Četke slobodno rotiraju oko vertikalne ose i prinudno osciliraju gore-dole. Tako „uronjene“ u samoj biljci izazivaju opadanje zrelih plodova. Oni se dalje usmeravaju na transportne trake sa jedne i druge strane, a strujanjem vazduha vrši se odstranjivanje nečistoća, te čisti plodovi završavaju u gajbi.

Slika 1: Samohodni kombajn za berbu maline

Izvor: SZR „Elektronik“

Razvoj voćarstva u Srbiji uslovljen je prozvodnjom i plasmanom visoko kvalitetnog voća. Da bi poljoprivredni proizvođači mogli ponuditi tržištu vrhunski proizvod, neophodno je sprovesti berbu na odgovarajući način (Funt *et al.*, 1998). Iz tog razloga, kvalitet mašinski ubranih malina upoređen je sa onim ručno ubranim (Tab. 1).

Tabela 1: Razlika u kvalitetu ručno i mašinski ubranih malina

Način berbe	Čvrstina ploda (N)[3]	Rastvorljive suve materije (%)[4]	Titracijska kiselost (%)[5]	Vrednosti refleksije boje[6]		
				L	Θ	Chroma
Ručna	1,14	11,3	0,74	16,2	12,1	15,8
Mehanička	1,25	11,5	0,71	15,9	12,1	14,5

Izvor: Funt *et al.*, 1998.

Da bi se izvršilo poređenje troškova ručne i mehanizovane berbe voća neophodno je najpre utvrditi eksploatacione troškove rada kombajna za ubiranje maline. U tabeli 2 dat je pregled eksploatacionih troškova kombajna za ubiranje maline. Prilikom utvrđivanja eksploatacionih troškova rada kombajna uzeto je da je nabavna cena 45.000 €. Prilikom kupovine kombajna podignut je kredit u iznosu pune nabavne cene, sa kamatom od 6% i periodom otplate od 5 godina. Analizirani kombajn je osiguran, pri čemu premijska stopa iznosi 2,2%. Procenjeni ekonomski vek kombajna je 8.000 časova rada i predviđena je njegova godišnja upotreba od 800 časova. Za berbu maline specijalizovanim kombajnom neophodna su tri radnika.

Tabela 2: Eksploatacioni troškovi kombajna za ubiranje malina

Elementi troškova	Troškovi rada kombajna			
	(€/h)	(RSD/h)	(€/ha)	(RSD/ha)
Troškovi amortizacije	5,63	675,6	3,52	1.080,96
Troškovi održavanja	13,65	1.638	8,53	2.620,8
Troškovi energije	3,65	438	2,28	700,8
Troškovi kamata i osiguranja	3,27	392,4	2,04	627,84
Troškovi radne snage	3,75	450	2,34	720
Ukupno	29,95	3.594	18,72	5.750,4

Izvor: Autori na osnovu podataka preuzetih iz SZR „Elektronik"

[3] Čvrstina maline (sila u Njutnima koja je potrebna da se zatvori centralna šupljina) određena je uz pomoć Ametec AccuForce II (model ML-4432-5) koji je opremljen sa ravnom sondom.
[4] Izmeren je nivo rastvorljivosti suvih materija uz pomoć refraktometrije.
[5] Izmeren je nivo titracijske kiselosti plodova.
[6] Parametri refleksije boja izračunati su na Hunter Color Difference metra. Vrednosti ukazuju na sledeće: L ukazuje na svetlinu/tamnoću (0 = čisto crno, 100 = čisto belo); Θ (ugao nijanse) ukazuje na nijansu koja je prikazana u stepenima u krugu (0° = čisto crvena, 90° = čisto žuta, 180° = čisto zelena, 270° = čisto plava, 360° = čisto crvena); chroma prikazuje relativni intenzitet boja (visoke vrednosti ukazuju na živopisne boje).

Kod proračuna troškova ručne berbe najpre je izračunato koliko je radnika potrebno da bi se berba jednog hektara završila za jedan radni dan, odnosno za 8 časova. Zatim je određena cena radnog sata, i na kraju ukupni troškovi ručnog branja.

Tabela 3: Troškovi ručne berbe po hektaru

Voćna vrsta	Potreban broj radnika	Radno vreme (h)	Cena radnog sata		Troškovi branja	
1	2	3	4		5 = 2·3·4	
Malina	106	8	RSD/h	€/h	RSD	€
			180	1,5	152.640	1.272

Izvor: Proračun autora na osnovu Keserović i sar., 1999.

Kada se uporede troškovi berbe primenom radne snage, odnosno troškovi ručne berbe (152.640 RSD/ha) sa troškovima mehaničke berbe (5.750,40 RSD/ha) dolazi se do zaključka da je mehanizovana berba primenom kombajna jeftinija za čak 26,54 puta. Osim značajno manjih troškova berbe, primena kombajna ima i brojne druge prednosti u odnosu na ručnu berbu. U tabeli 4 prikazane su najvažnije prednosti, kao i nedostaci mehanizovane berbe.

Tabela 4: Prednosti i nedostaci mehanizovane berbe maline

PREDNOSTİ	NEDOSTACİ
1. Veća mikrobiološka ispravnost, 2. Mnogo manji broj radnika, 3. Kraći period berbe, 4. Omogućava povećanje površina pod voćnjacima, 5. Mogućnost berbe tokom noći, 6. Visoka efikasnost branja (stepen otrešenosti stabla preko 95%), 7. Beračem se lako rukuje, 8. Ne postoji štetno dejstvo na stablo, 9. Isključena je mogućnost povreda usled pada sa merdevina, itd.	1. Neujednačenost dozrevanja plodova, 2. Voće ubrano mehanizovanim putem uglavnom se koristi za industrijsku preradu zbog malo lošijeg kvaliteta, 3. Visoka nabavna cena mašine.

Izvor: Calvin and Martin, 2010; Huffman, 2012.

Ekonomska efikasnost je relativno merilo ekonomskog uspeha. Ona se određuje stavljanjem u odnos apsolutnih proizvodnih rezultata i troškova. Efikasnost se ne izražava u novčanim jedinicama, već u procentima ili koeficijentom (Novković i

Šomođi, 2001). Osnovne ekonomske kategorije kojima se meri ekonomska efikasnost su produktivnost rada, ekonomičnost i rentabilnost. Za izračunavanje ovih pokazatelja korišćeni su ekonomski parametri koji su prikazani u tabeli 5.

Tabela 5. Izračunavanje osnovnih ekonomskih parametara

Prosečan prinos maline (kg/ha)	Cena maline (RSD/kg)	Vrednost proizvodnje (RSD)	Troškovi ručne berbe (RSD/ha)	Troškovi mehaničke berbe (RSD/ha)	Dobit (RSD/ha)
1	2	1 x 2 = 3	4	5	4 - 5 = 6
5.120	134	686.080	152.640	5.750	146.890
-	(€/kg)	(€)	(€/ha)	(€/ha)	(€/ha)
-	1,12	5.717	1.272	47,92	1.224

Izvor: Proračun autora

S obzirom da je dnevni učinak jednog berača 48,32 kg, produktivnost rada (formula 1) ručne berbe maline iznosi 6,04 kg/h. Sa druge strane, dnevni učinak kombajna iznosi 5 ha, odnosno 25.600 kg (proizvod prosečnog prinosa maline po hektaru i dnevnog učinka kombajna), te je produktivnost kod mehanizovane berbe 3.200 kg/h. Ovako izračunata produktivnost rada pokazuje ostvareni obim proizvodnje po jednom času uloženog rada.

Koeficijent ekonomičnosti (formula 2) kod ručne berbe iznosi 4,49, dok kod mehanizovane berbe iznosi 119,32. Ovako dobijeni koeficijenti ekonomičnosti pokazuju koliko je dinara vrednosti proizvodnje ostvareno na dinar troškova ručne, odnosno mehanizovane berbe.

Ekonomska opravdanost mehanizovane berbe maline dokazana je i činjenicom da se je ovaj vid berbe rentabilniji za 21,41% u odnosu na ručnu berbu, obzirom da su troškovi mehaničke berbe daleko manji u odnosu na troškove ručne berbe (formula 3).

ZAKLJUČAK

Za racionalno ubiranje maline preporučuje se korišćenje adekvatne mehanizacije, konkretno specijalizovanih kombajna za ubiranje maline i kupine. Ovakvim načinom ubiranja znatno se smanjuje agrotehnički rok, zavisnost od ljudske radne snage, kao i troškovi branja, pri čemu će proizvođači biti u mogućnosti da voće gaje na znatno većim površinama.

Kao što su prethodni proračuni pokazali, troškovi mehanizovane berbe maline su 26,54 puta manji u odnosu na troškove ručne berbe. Izračunati pokazatelji ekonomske efikasnosti takođe jasno govore u prilog činjenici da je primena mehanizovane berbe apsolutno ekonomski opravdana, te da kao takva predstavlja budućnost i nužnost voćarske proizvodnje, kako u svetu, tako i kod nas.

LITERATURA

Calvin Linda and Martin P. (2010): The U.S. Produce Industry and Labor, Facing the Future in a Global Economy, Economic Research Report, Number 106, United States Department of Agriculture.

Funt R. C., Wall T. E. and Scheerens J. C. (1998): Yield, Berry Quality, and Economics of Mechanical Berry Harvest in Ohio, Bulletin, Fruit crops: A summary of research 1998, Research circular 299-99, The Ohio State University.

Huffman W. E. (2012): The status of labor-saving mechanization U.S fruit and vegetable harvesting, Choices – The magazine of food, farm and resource issues, Agrucultural and applied economics association, USA.

Keserović Z., Gvozdenović D., Grgurević V. i Živanović M. (1999): Proizvodnja voća na malim površinama, Savez učeničkih zadruga Srbije, Beograd, Poljoprivredni fakultet, Novi Sad, str. 55-68.

Mika A., Wawrzyńczak P., Buler Z., Dorota Konopacka, Konopacki P., Krawiec A., Białkowski P., Barbara Michalska, Plaskota M. and Gotowicki B. (2012): Mechanical harvesting of plums for processing with a continuously moving combine harvester, Research Institute of Horticulture, Journal of Fruit and Ornamental Plant Research, vol. 20, p. 29-42, Skierniewice, Poland.

Novković N. i Šomođi Š. (2011): Organizacija u poljoprivredi, Poljoprivredni fakultet, Novi Sad, 2001, str. 89-92.

Urošević M., Radojević R., Petrović D. i Bižić Milica (2011): Opravdanost uvođenja mehanizovane berbe maline u Srbiji, Poljoprivredna tehnika, br. 3, Univerzitet u Beogradu, Poljoprivredni fakultet, Beograd, str. 79-86.

Veljić M., Mladenović N., Marković D. i Simonović V. (2009): Optimizacija parametara tehničkih rešenja za mašinsko branje koštičavog i jagodičastog voća, Poljoprivredna tehnika, br. 3, Univerzitet u Beogradu, Poljoprivredni fakultet, Beograd, str. 85-94.

Veljković Biljana, Petrović S., Leposavić A., i Glišić I. (2006): Profitabilnost proizvodnje maline na području Srbije. Ekonomika poljoprivrede, Vol. 53, 1013-1022.

Živković D. i Veljić M. (2011): Prilog određivanju ekonomičnosti trešenja višanja i šljiva, Poljoprivredna tehnika, br. 3, Univerzitet u Beogradu, Poljoprivredni fakultet, Beograd, str. 69-78.

ECONOMIC EFFICIENCY OF GREENHOUSE VEGETABLE PRODUCTION IN THE SARAJEVO CANTON*

EKONOMSKA EFIKASNOST PLASTENIČKE PROIZVODNJE U KANTONU SARAJEVO

Emir Bećirović[1], Sabahudin Bajramović[1], Anđelka Stojanović[1]

Originalni naučni rad

Rezime

Plastenička proizvodnja povrća posljednjih godina ima značajnu ekspanziju na području BiH. Mala gazdinstva karakteristična za BiH i visoka profitabilnost ovog tipa proizvodnje su glavni razlozi za sve češće usvajanje plasteničke proizvodnje na porodičnim gazdinstvima. U Kantonu Sarajevo je također sve veći broj proizvođača koji se bave prozvodnjom povrća u zaštićenom prostoru, dok su ekonomska istraživanja ovih porodičnih gazdinstava dosta skromna. Sve veći značaj proizvodnje povrća u zaštićenom prostoru i nedostatna istraživanja ovog tipa gazdinstava su glavni motiv izrade ovoga rada.
Osnovni izvor istraživačke građe za utvrđivanje proizvodno-ekonomskih rezultata porodičnih gazdinstava bili su anketni upitnici. Kako bi se provelo anketiranje na relevantnom uzorku, odabrano je 15 proizvodnih jedinica čija se veličina, mjerena veličinom zaštićenog prostora, kretala od 100 do 900 m^2. Sva gazdinstva se nalaze na području Kantona Sarajevo. Rezultati su prikazani kroz ukupnu ostvarenu neto dobit na gazdinstvu, te kroz ostvareno pokriće dvije dominantne proizvodnje u plastenicima (paradajz i špinat). Kako bi se što preglednije prikazali rezultati istraživanja, gazdinstva su podijeljena u dvije grupe: grupa gazdinstava do 300 m^2 plasteničke površine i grupa gazdinstava preko 300 m^2 plasteničke površine.
Kada se posmatra pokriće ostvareno po 100 m^2 proizvodnje paradajza bolje rezultate su ostvarila gazdinstva s manjom plasteničkom površinom sa 2.709 KM u odnosu na gazdinstva sa većom plasteničkom površinom, gdje je ovo pokriće iznosilo 2.578 KM. Ostvareno pokriće po 100 m^2 u proizvodnji špinata kod manje grupe gazdinstava iznosi 577 KM, dok je druga grupa gazdinstava ostvarila 515 KM.

Ključne riječi: *Kanton Sarajevo, plastenici, pokriće, dobit, povrće*

---

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Poljoprivredno-prehrambeni fakultet Univerziteta u Sarajevu

Summary

Greenhouse vegetable production has been expanding significantly in recent years in Bosnia and Herzegovina. Small farms that are characteristic for BiH and high profitability of this type of production are the main reasons behind the fact that greenhouse production has been increasingly adopted on family farms. In the Canton of Sarajevo there is also a growing number of producer involved in the production of vegetables in greenhouses, while economic research on these family farms has been rather modest. The growing importance of greenhouse vegetable production and lack of research on these types of farms are the main motives behind this work.

The main source of research material for determining the production and economic performance of family farms are questionnaires. To carry out the survey on a relevant sample, fifteen production units whose sizes of greenhouses ranged from 100 to 900 m^2 were selected. All are located in the Sarajevo Canton. The results are shown through the net profit of farms and the achieved gross margin of two dominant products in greenhouses (tomato and spinach). In order to show more clearly the results of the research, farms were divided into two groups: a group of farms with up to 300 m^2 of the greenhouse area and a group of farms with over 300 m2 of the greenhouse area.

Regarding the gross margin for 100 m^2 of tomato better results were achieved by farms with smaller greenhouse areas with 2,709 KM compared to the farms with a larger greenhouse surface, where the gross margin amounted to 2,578 KM. The gross margin for 100 m^2 in the production of spinach on smaller farms amounted to 577 KM, while for the other group it was 515 KM.

Key words: *Canton Sarajevo, greenhouse, gross margin, profit, vegetables*

UVOD

Relativno povoljni klimatski uslovi, niske cijene zemljišta i drugih faktora proizvodnje u odnosu na regiju, te preko 20% stanovništva oficijelno uključenog u poljoprivrednu proizvodnju bi mogli biti početni katalizatori ruralnog razvoja Bosne i Hercegovine (Zadružni savez BiH). U okviru poljoprivrede, uz stočarsku i voćarsku proizvodnju, povrtlarska proizvodnja, sa naglaskom na plasteničku proizvodnju, bi trebala zauzimati jednu od vodećih pozicija razvoja. Bosna i Hercegovina posjeduje relativno dobre prirodne potencijale za razvoj povrtlarske proizvodnje, međutim, ponekad se suočava sa otežavajućim faktorima, kao što su nepovoljni vremenski uslovi (visoke ili niske temperature, kasni proljetni ili rani jesenji mrazevi, deficit ili suficit padavina) U tom kontekstu, plastenička proizvodnja kao oblik uzgoja bilja sa djelimično kontrolisanim uslovima proizvodnje može biti jedno od rješenja za prevazilaženje ovih problema (Bećirović, 2015). U okviru povrtlarske proizvodnje, koja je u cjelini u porastu na nivou BiH, snažno jača i njena proizvodnja u zaštićenim prostorima. Mali raspoloživi zemljišni resursi su glavni razlog zbog čega se porodična gazdinstva odlučuju za visoko profitabilne proizvodnje po jedinici zemljišne površine, kao što su proizvodnja jagodičastog voća i svakako proizvodnje povrća u zaštićenom prostoru (Bećirović *et al.*, 2016). Tuneli, plastenici, lijehe, staklenici i drugi različiti agrotekstilni, termoselektivni materijali sa svojim tehnološkim procesima predstavljaju inovativnu proizvodnju u poljoprivredi. Istraživanja koja je obavio Hanić (2006) pokazuju da se za ovu proizvodnju mogu koristiti i površine koje skoro da imaju bonitet od VI do VIII klase. Dakle, najlošija tla koja se isključuju iz proizvodnje ili zahtijevaju jako visoka ulaganja za njihovo privođenje kulturi (krš, kamenjari, odlagališta, kontaminirane površine, teška, zbijena, hidrogenizirana, zaslanjena ili slana zemljišta). Ova zemljišta zahvaljujući novim tehnologijama mogu se potpuno supstituisati kao proizvodni mediji. Autor također tvrdi, da količine prirodnih padavina, ukoliko se kolektiraju i akumuliraju, uz racionalno upravljanje i korištenje mogu biti dovoljne da pokriju potrebe u vodi za cjelokupnu godišnju proizvodnju. To znači da količine padavina (1400-1500 mm/m^2) mogu biti dovoljne za konstantnu proizvodnju. Đurovka *et al.* (2006) smatraju da se najekonomičnija proizvodnja ostvaruje u zaštićenoj bašti, koja je zbir više tipova objekata staklenika, plastenika, različitih tunela uz korištenje agrotekstila, sa i bez grijanja, koji omogućuju cjelogodišnji ciklus proizvodnje od jeseni do jeseni. Prema istom autoru blizina tržišta, odnosno mogućnost dobrog i brzog transporta je odlučujući faktor za ekonomičnost proizvodnje. Povoljni tržišni uslovi mogu eliminisati ostale nepovoljne uslove za prizvodnju. Ekonomičnost proizvodnje je uslovljena intenzivnom proizvodnjom, brojem gajenih vrsta u toku godine ili visokim prinosima i kvalitetom, a prema zahtjevu tržišta. Pun ekonomski efekat ostvaruje se robnom proizvodnjom organizovanih proizvođača ili sopstvenom proizvodnjom, a za poznato tržište. Plastenička proizvodnja povrća na godišnjem nivou, obezbjeđuje značajno najveći profit, u odnosu na brojne vidove proizvodnje hrane. Ono što bi povrtlarsku proizvodnju učinilo još profitabilnijom je podizanje plasteničke proizvodnje sa orijentacijom na ranu proizvodnju paradajza, paprike, krastavca i dr. (Ministarstvo poljoprivrede, vodoprivrede i šumarstva Republike Srpske, 2009) Proizvodnja u zaštićenim prostorima (staklenici, plastenici, različiti modeli tunela) predstavlja najintenzivniji oblik proizvodnje povrća. U interesu postizanja viših cijena, poljoprivredni proizvođači su oduvijek nastojali da se sa svojim proizvodima na tržištu ranije pojavljuju ili proizvodnju tempiraju izvan glavne sezone. Zaštićeni prostori omogućavaju proizvođačima organiziranje visoko akumulativne proizvodnje tokom cijele godine. Proizvodnja povrća u zaštićenim prostorima ima veliki biološki i ekonomski značaj. Ovaj vid proizvodnje osigurava svježe povrće u jesenskom, zimskom i rano proljetnom periodu, zatvarajući tako sa ljetnom proizvodnjom puni godišnji ciklus produkcije. Prinosi glavnih kultura (paradajz, paprika, krastavac, salata) su veći 3-5 puta od onih koji se postižu na otvorenim površinama. Istraživanja obavljena posljednjih godina pokazuju dobitak u proizvodnji naprijed navedenih kultura na otvorenim površinama, i komparaciji sa površinama pod pšenicom veći su od 30 do 80 puta. Odnos je daleko veći kada se porede dobiti u zaštićenim prostorima. Zaštićeni prostori omogućavaju organizaciju intenzivne i egzistencijalno sigurne proizvodnje na malim površinama. To je posebno relevantno za mediteransko područje, koje oskudjeva obradivim poljoprivrednim površinama (Karić, 2015).

Prema Kurtoviću i Lokvančić (2011) posljednjih godina, ova se proizvodnja sve više širi na cijelom prostoru BiH, a posebno je značajna ekspanzija u kontinentalnom području. Bajramović *et al.* (2011) navode kako je proizvodnja u zaštićenom prostoru važna i brzorastuća komponenta poljoprivredne industrije razvijenih zemalja. U BiH ova je proizvodnja još uvijek u fazi razvoja. Međutim, u primjetnom je porastu kao i sve veći zahtjevi stanovništva za svježim povrćem.

Dakle, ekspanzija plasteničke proizvodnje u Bosni i Hercegovini je evidentna realnost, a tome su doprinijeli razni međunarodni programi, koji su promovisali ovaj vid proizvodnje, osiguravali povoljne kredite i edukaciju poljoprivrednih proizvođača. Do unazad 20-30 godina, ova proizvodnja je bila locirana samo u južnom dijelu BiH sa zastupljenom mediteranskom klimom, u dolini rijeke Neretve, te njenih pritoka. Plastenička proizvodnja se uspješno proširila i na kontinentalne dijelove Bosne i Hercegovine, te i dalje ima osjetan i zavidan trend rasta. Ako se ovome doda postojanje određenih mjera podrške pojedinih kantona kroz davanje poticaja ovoj proizvodnji, te slaba mogućnost zapošljavanja u drugim privrednim granama, onda je i bilo za očekivati da se sve veći broj poljoprivrednih gazdinstava okreće upravo plasteničkoj proizvodnji kao osnovnoj privrednoj djelatnosti.

Kanton Sarajevo putem novčanih podsticajnih sredstava vrši podsticanje kupovine novih plastenika, a sve u cilju motivisanja poljoprivrednih proizvođača da se više orjentišu na plasteničku proizvodnju povrća. Plastenička proizvodnja povrća bi imala puno veći efekat, kako u prinosu tako i finansijski, pogotovo ako se ima u vidu da su raspoložive zemljišne parcele poljoprivrednih proizvođača relativno male i da je obim proizvodnje povrća na otvorenom ograničen vremenskim uslovima. Kao i u ostatku BiH i u ovom kantonu je također sve veći broj proizvođača koji se bave prozvodnjom povrća u zaštićenom prostoru. Plastenička proizvodnja na ovom području ima još jednu značajnu prednost koja se ogleda u blizini velikog tržišta (grad Sarajevo) čije su potrebe za svježim voćem i povrćem značajne. Ipak, ekonomska istraživanja ovih porodičnih gazdinstava su dosta skromna i nedovoljna. Sve veći značaj proizvodnje povrća u zaštćenom prostoru i nedostatna istraživanja ovog tipa gazdinstava su glavni motiv izrade ovoga rada.

Ovaj rad ima za cilj da se izvrši uvid u postojeće stanje i korištenje raspoloživih resursa koja se odnose na proizvodnju povrća u zaštićenom prostoru (plasteniku) na području Kantona Sarajevo. Na taj način doći će se do dokumentovanih podloga za utvrđivanje ekonomike proizvodnje povrća u plastenicima, te njihovog značaja na socijalni i ekonomski položaj poljoprivrednih gazdinstava.

MATERIJAL I METODE

Osnovni izvor istraživačke građe za utvrđivanje proizvodno-ekonomskih rezultata porodičnih gazdinstava bili su anketni upitnici. Prilikom izbora jedinica posmatranja vodilo se računa da uzorak bude što reprezentativniji, kako bi se što realnije prikazalo stvarno stanje na datom području istraživanja. To je značilo odabir porodičnih gazdinstava različite veličine mjerene veličinom plasteničkog prostora, te različite teritorijalne pripadnosti na istraživanom području Kantona Sarajevo. Ukupno je odabrano 15 proizvodnih jedinica istraživanja, čija se veličina mjerena veličinom zaštićenog prostora, kretala od 100 do 900 m^2 površine pod plastikom. U svrhu izrade ovoga rada anketni upitnik je sadržavao sekcije o prosječnim prinosima, prihodima, varijabilnim i fiksnim troškovima, što je vodilo do dobijanja rezultata o ukupnoj dobiti ostvarenoj na posmatranim gazdinstvima.

Svi podaci u ovome radu su dobijeni kroz intervju sa vlasnicima porodičnih farmi i uneseni su u anketne upitnike. Kada su upitnici kompletirani, prikupljeni podaci su obrađeni kako bi se dobile odgovarajuće vrijednosti i pokazatelji. Za svu numeričku obradu podataka korišten je kompjuterski program Microsoft Excel 2010.

Rezultati su prikazani kroz ukupnu ostvarenu neto dobit na gazdinstvu, te kroz ostvareno pokriće dvije dominantne proizvodnje u plastenicima (paradajz i špinat). Kako bi se što preglednije prikazali rezultati istraživanja, gazdinstva su podijeljena u dvije grupe: grupa gazdinstava do 300 m^2 plasteničke površine i grupa gazdinstava preko 300 m^2 plasteničke površine. Rezultati su posebno prikazani za obje ove grupe gazdinstava, te su dati i prosječni rezultati za sva posmatrana gazdinstva. Broj istraživanih gazdinstava koji raspolažu sa manje od 300 m^2 plasteničke površine je bio osam, a gazdinstava koji imaju preko 300 m^2 plasteničke površine bilo je sedam.

U ovome radu su se koristili pokazatelji koji se odnose na 100 m^2 površine staklenika, promatrajući prinos, prihod i troškove po jedinici proizvodnje. Prihod uključuje sve prihode od proizvodnje u plastenicima, a troškovi su svrstani u dvije odvojene skupine: varijabilni i fiksni troškovi. Konačna dobit na farmi obuhvaća sve prihode i rashode poljoprivrednog gospodarstva u smislu biljne i stočarske proizvodnje.

Jedan od načina prikazivanja dobivenih rezultata u ovom radu jeste pokriće (eng. gross margin) ostvareno pojedinačnom proizvodnjom. Prema Kay i Edwards (1999), pokriće predstavlja razliku između prihoda i varijabilnih troškova po jedinici mjere (1 ha ili 1 grlo) pojedinačnih proizvodnih linija poljoprivrednog gazdinstva. Ono služi za pokrivanje fiksnih troškova i ostvarivanje dobiti nakon što se plate varijabilni troškovi. Ovdje će se prikazati ostvareno pokriće proizvodnjom paradajza i špinata, kao dvije najznačajnije kulture na istraživanim gazdinstvima.

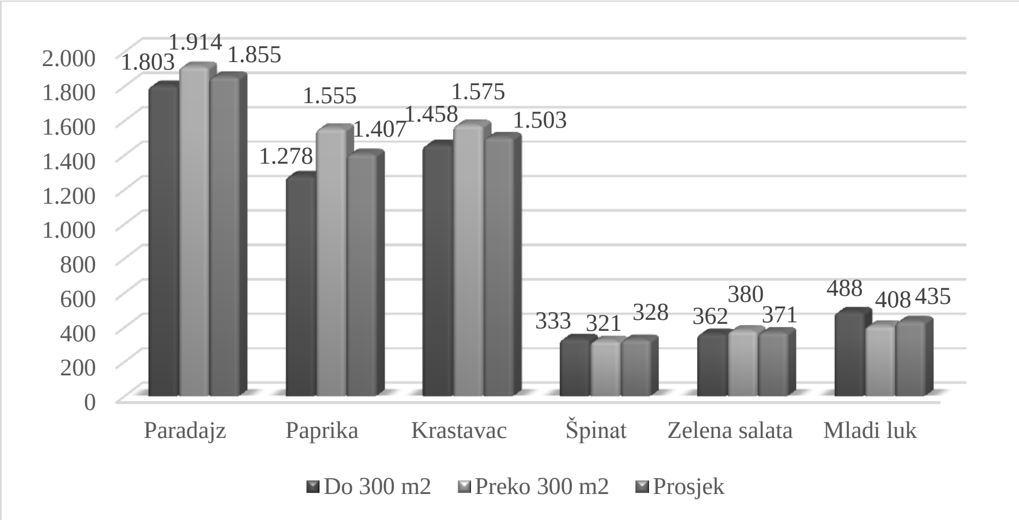
Neto dobit farme je jedan od najvažnijih pokazatelja ekonomskog uspjeha gazdinstva. Predstavlja razliku ukupnih prihoda i ukupnih troškova gazdinstva. Neto dobit gazdinstva pokazuje rezultat uloženog vlastitog rada i upravljanja investicijama na gazdinstvu, odnosno predstavlja visinu povrata na investirani kapital u gazdinstvo, bez obzira da li je kapital posuđen ili ne (Dillon i Hardaker, 1993). Izračunava se tako što se od zbira pokrića za pojedine linije proizvodnje na gazdinstvu oduzmu fiksni troškovi.

REZULTATI ISTRAŽIVANJA

Ukupno raspoloživo zemljište svih 15 istraživanih gazdinstava iznosilo je 10,69 ha. Od ovoga, poljoprivredno zemljište je bilo rasprostranjeno na površini od 10,18 ha, odnosno 95,23%. Obradivo zemljište zauzimalo je 9,78 ha, odnosno 91,45% ukupnog zemljišta. Najveći udio u ukupnim površinama se odnosi na oranice i vrtove sa 4,84 ha, što iznosi 45,28%, a od toga je pod plastenicima 0,54 ha, odnosno 5,05%. Prosječno gazdinstvo je veličine 0,71 ha, pri čemu raspolaže sa 0,68 ha poljoprivrednog zemljišta. Gazdinstva koja su imala preko 300 m^2 plasteničkih površina su znatno veća (1,06 ha) u odnosu na gazdinstva iz grupe do 300 m^2 (0,41 ha). Manja grupa gazdinstava su prosječno imala 180 m^2 plastenika, nasuprot tri puta većoj površini plastenika u iznosu od 560 m^2 druge istraživane grupe gazdinstava.

Najdominantnija kultura koja se uzgajala u zimskom periodu jeste špinat koji je u prosjeku zauzimao 223 m^2 plastenika, od prosječmo raspoloživih 360 m^2, što predstavlja 62,04%. U toku ljeta u proizvodnji je najviše bio zastupljen paradajz sa 205 m^2, odnosno 57,04% od prosječno raspoloživih plasteničkih površina. Relativni udio pojedinih kultura u ukupnim raspoloživim površinama pojedine grupe posmatrnih gazdinstava je bio približno ujednačen.

Prosječni ostvareni prinosi važnijih povrtlarskih kultura u plastenicima na posmatranim gazdinstvima dati su na grafikonu 1. Prinos paradajza iznosio je 1.855 kg/100 m^2, paprike 1.407 kg/100 m^2, krastavca 1.503 kg/100 m^2, špinata 328 kg/100 m^2, zelene salate 371 kg/100 m^2 i mladog luka 435 kg/100 m^2. Najveća razlika u prinosima između dvije posmatrane grupe gazdinstava se pojavljuje kod proizvodnje paprike čiji su prinosi u prvoj posmatranoj grupi 1.278 kg/100 m^2, u odnosu na 1.555 kg/100 m^2 koliko iznose u drugoj grupi poljoprivrednih gazdinstava. Razlika se javlja kod proizvodnje paradajza, gdje su prosječni prinosi veći za 111 kg/100 m^2 i krastavca za 107 kg/100 m^2 u korist gazdinstava sa većom plasteničkom površinom. Prosječni prinosi špinata i zelene salate su ujednačeni kod obje posmatrane grupe i razlika gotovo da i ne postoji, dok prosječni prinosi mladog luka kod prve grupe iznose 488 kg/100 m^2 i u neznatnoj su prednosti u odnosu na drugu grupu istraživanih gazdinstava (408 kg/100 m^2).



Grafikon 1: Prosječni prinosi važnijih povrtlarskih kultura u plastenicima po istraživanim grupama gazdinstava (kg/100 m^2)

Figure 1: Average yield of significant vegetable cultures in greenhouses depending on the size of the farm (in kg/100 m^2)

U tabeli 1 je predstavljeno ostvareno pokriće proizvodnjom paradajza na posmatranim gazdinstvima po jedinici površine (100 m^2). Utvrđeno je da veće prihode po jedinici površine ostvaruju gazdinstva sa manjom plasteničkom površinom (2.996 KM/100 m^2) u odnosu na gazdinstva sa većom plasteničkom površinom (2.800 KM/100 m^2).

Tabela 1: Ostvareno pokriće proizvodnjom paradajza na posmatranim gazdinstvima zavisno od veličine gazdinstva (KM/100 m^2)

Table 1: Gross margin of tomato production on observed farms depending on the size of the farm (KM /100 m^2)

Opis	Gazdinstva prema veličini zaštićenog prostora		Prosjek
	Do 300 m^2	**Preko 300 m^2**	
A) PRIHODI	**2.996**	**2.800**	**2.904**
B) VARIJABILNI TROŠKOVI			
4. Sjeme	153	121	138
5. Đubrivo	64	65	64
6. Zaštitna sredstva	9	4	7
7. Iznajmljeni mašinski rad	0	0	0
8. Ostali troškovi	61	32	48
Svega (B)	**287**	**222**	**257**
C) POKRIĆE (A-B)	**2.709**	**2.578**	**2.648**

Izvor: Vlastita istraživanja

Prosječno gazdinstvo je imalo prihode od 2.904 KM/100 m^2. Gazdinstva prve posmatrane grupe su imala i veće pokriće u proizvodnji paradajza po 100 m^2 plastenika za 131 KM u odnosu na drugu grupu istraživanih gazdinstava. Razlozi ovome se nalaze u višim prodajnim cijenama.

Na istraživanim gazdinstvima prihod ostvaren proizvodnjom špinata iznosio je 631 KM/100 m^2, troškovi su iznosili 83 KM/100 m^2, dok je ostvareno pokriće bilo 548 KM. Nešto više prihode imaju gazdinstva iz prve grupe sa 667 KM/100 m^2 u odnosu na prihode druge istraživane grupe gazdinstava sa iznosom od 589 KM/100 m^2. Troškovi su neznatno veći kod prve posmatrane grupe po 100 m^2, ali se to nije odrazilo na pokriće koje je za 62 KM/100 m^2 veće nego u drugoj istraživanoj grupi gazdinstava.

Tabela 2: Ostvareno pokriće proizvodnjom špinata na posmatranim gazdinstvima zavisno od veličine gazdinstva (KM/100 m^2)
Table 2: Gross margin of spinach production on observed farms depending on the size of the farm (KM /100 m^2)

Opis	**Gazdinstva prema veličini zaštićenog prostora**		**Prosjek**
	Do 300 m^2	**Preko 300 m^2**	
A) PRIHODI	**667**	**589**	**631**
B) VARIJABILNI TROŠKOVI			
4. Sjeme	25	17	21
5. Đubrivo	29	33	31
6. Zaštitna sredstva	6	3	5
7. Iznajmljeni mašinski rad	0	0	0
8. Ostali troškovi	31	21	26
Svega (B)	**90**	**74**	**83**
C) POKRIĆE (A-B)	**577**	**515**	**548**

Izvor: Vlastita istraživanja

Ukupni prihodi prosječnog gazdinstva su iznosili 19.880 KM, od čega je od plasteničke prozvodnje ostvareno 10.847 KM, proizvodnje na otvorenom 3.157 KM, voćarske proizvodnje 4.285 KM, te stočarske 1.591 KM. Prihodi gazdinstava druge grupe iznose 27.980 KM, pri čemu plastenička proizvodnja učestvuje sa 16.504 KM, a proizvodnja na otvorenom 5.185 KM, voćarska 3.482 KM, te stočarska proizvodnja sa 2.809 KM. U prvoj grupi gazdinstava prihodi su bili 12.793 KM, od čega su najvažniji iz plasteničke proizvodnje i iznose 5.898 KM. Značajan izvor prihoda kod ove posmatrane grupe su prihodi iz voćarske proizvodnje sa ukupnim iznosom od 4.988 KM. Varijabilni troškovi su iznosili 6.647 KM, pri čemu najveću stavku čini sjeme, odnosno sadnice i đubriva. Ovi troškovi kod druge grupe gazdinstava iznose 8.929 KM, dok kod prve grupe gazdinstava varijabilni troškovi imaju vrijednost od 4.650 KM. U kategoriji fiksnih troškova najznačajniji su procijenjeni troškovi rada članova porodice koji iznose 8.840 KM što čini značajan dio od 11.295 KM kolika je vrijednost fiksnih troškova. Fiksni troškovi druge grupe gazdinstava imaju iznos od 15.240 KM, dok kod prve posmatrane grupe oni iznose 7.844 KM. Ukupni troškovi svih gazdinstava su 18.024 KM, pri čemu kod druge grupe gazdinstava iznose 24.345 KM. Ukupni troškovi prve istraživane grupe imaju iznos od 12.493 KM. Na svim gazdinstvima ostvaren je pozitivan rezultat iz upravljanja i investicija u prosjeku sa 2.122 KM. Grupa gazdinstva sa većom plasteničkom površinom ostvarila je dobitak od 4.157 KM, dok je kod gazdinstva sa manjom plasteničkom površinom dobitak iznosio 342 KM.

Tabela 3: Poslovni uspjeh gazdinstva iskazan kroz dobit iz upravljanja i investicija i neto dobit farme

Table 3: Business success of the farm shown through profit from the management and investment, and net farm profit (average per farm in KM)

Opis	**Gazdinstva prema veličini zaštićenog prostora**		**Prosjek**
	Do 300 m^2	**Preko 300 m^2**	
A) PRIHODI IZ POJEDINAČNIH			
1. Plastenička proizvodnja	5.898	16.504	10.847
2. Biljna proizvodnja na otvorenom	1.383	5.185	3.157
3. Voćarska proizvodnja	4.988	3.482	4.285
4. Stočarska proizvodnja	525	2.809	1.591
SVEGA (A)	**12.793**	**27.980**	**19.880**
B) VARIJABILNI TROŠKOVI			
1. Plastenička proizvodnja	800	1.741	1.239
2. Biljna proizvodnja na otvorenom	944	3.537	2.154
3. Voćarska proizvodnja	2.533	1.859	2.218
4. Stočarska proizvodnja	374	1.791	1.035
UKUPNO VARIJABILNI TROŠKOVI	**4.650**	**8.929**	**6.647**
C) UKUPNO POKRIĆE (A-B)	**8.142**	**19.051**	**13.233**
D) OSTALI PRIHODI GAZDINSTVA	44	346	185
E) TROŠKOVI OSTALIH DJELATNOSTI	27	171	94
F) FIKSNI TROŠKOVI	7.844	15.240	11.295
UKUPNO TROŠKOVI (B+E+F)	**12.493**	**24.345**	**18.024**
G) DOBIT IZ UPRAVLJANJA I INVESTICIJA	**342**	**4.157**	**2.122**
Plus			
Vrijednost vlastitog rada članova porodice	6.413	11.614	8.840
H) NETO DOBIT FARME	**6.755**	**15.771**	**10.962**

Izvor: Vlastita istraživanja

Kada se doda vrijednost vlastitog rada članova porodice dobije se rezultat od 10.962 KM ostvarene dobiti po gazdinstvu, gdje je ona znatno veća kod druge posmatrane grupe 15.771 KM, dok kod prve grupe gazdinstava iznosi 6.755 KM.

ZAKLJUČAK

Prosječni prinosi paradajza na istraživanim gazdinstvima su iznosili 1.855 kg/100 m^2, paprike 1.407 kg/100 m^2, krastavca 1.503 kg/100 m^2, špinata 328 kg/100 m^2, zelene salate 371 kg/100 m^2 i mladog luka 435 kg/100 m^2. Posmatrajući ostvareno pokriće proizvodnjom paradajza na 100 m^2 bolje rezultate su ostvarila gazdinstva s manjom plasteničkom površinom sa 2.709 KM u odnosu na gazdinstva sa većom plasteničkom površinom, gdje je ovo pokriće iznosilo 2.578 KM. Ukupno pokriće u proizvodnji špinata kod prve grupe gazdinstava isnosi 694 KM, a 577 KM/100 m^2. Druga grupa gazdinstava ostvarila je pokriće od 1.508 KM, odnosno 515 KM/100 m^2. Na svim gazdinstvima ostvaren je pozitivan rezultat iz upravljanja i investicija u prosjeku sa 2.122 KM. Dobit kod gazdinstva sa većom plasteničkom površinom iznosi 4.157 KM dok na gazdinstvima sa manjom plasteničkom površinom ima vrijednost od 342 KM. Kada se doda vrijednost vlastitog rada članova porodice dobije se rezultat od 10.962 KM ostvarene dobiti po gazdinstvu, gdje je ona znatno veća kod druge posmatrane grupe 15.771 KM u odnosu na prvu grupu gazdinstava gdje iznosi 6.755 KM.

LITERATURA

Bajramović, S., Čustović, H., Bećirović, E., Žurovec, O. (2011): Studija, Mogućnosti proizvodnje u zaštićenom prostoru na području Općine Sanski Most, Poljoprivredno-prehrambeni fakultet Univerziteta u Sarajevu, Sarajevo, BiH.

Bećirović, E. (2015): Komparativna analiza proizvodno-ekonomskih rezultata plasteničkog uzgoja povrća u uslovima mediteranske i kontinentalne klime Bosne i Hercegovine, magistarski rad, Poljoprivredno-prehrambeni fakultet Univerziteta u Sarajevu.

Bećirović, E., Bajramović, S., Dragana Ognjenović, Merima Makaš, Kulelija, B. (2016): Economic efficiency of the green houses' vegetable production in the conditions of continental and mediterranean climate in Bosnia and Herzegovina, Works of the Faculty of Agriculture and Food Sciences University of Sarajevo, No 66/1, Sarajevo BiH.

Dillon, J. L., Hardaker, J. B. (1993): Farm menagemenet Research for Small Farmer Development, FAO Farm System Menagement Series, No 6, FAO, Rome, Italy.

Đurovka, M., Lazić, B., Bajkin, A., Potkonjak, A., Marković, V., Ilin, Ž., Todorović, V. (2006): Proizvodnja povrća i cveća u zaštićenom prostoru, Poljoprivredni fakultet Novi Sad, Poljoprivredni fakultet Banja Luka.

Hanić, E. (2006): Plastenička proizvodnja i razlozi njene ekspanzije, Stručno savjetovanje u Konjicu, Konjic, BiH.

Karić, L. (2015): Proizvodnja u plastenicima, Poljoprivredno-prehrambeni fakultet Univerziteta u Sarajevu, Neautorizovana predavanja, Sarajevo, BiH.

Kay, D. R., Edwards, W. M. (1999): Farm management, Fourth edition, WCB/MCGRAW-Hill, Boston, USA.

Kurtović, O., Lokvančić, L. (2011): Proizvodnja povrća i jagode u zaštićenim prostorima, Federalni zavod za poljoprivredu Sarajevo, Sarajevo

Ministarstvo poljoprivrede, vodoprivrede i šumarstva Republike Srpske (2009): Strategija razvoja poljoprivrede do 2015. godine, Banja Luka.

Zadružni savez BiH (2011): Mogućnosti i uslovi organizovanja zadruga sekundarnog reda u Bosni i Hercegovini, Studija izvodljivosti, CEDAR Sarajevo, Poljoprivredno-prehrambeni fakultet Sarajevo, Zadružni savez Slovenije, 2011, Sarajevo, BiH, str. 22-24.

TRANSFER OF RESEARCH RESULTS TO PRAXIS – THE CASE OF ISTRIA YOUNG POTATO*

TRANSFER ISTRAŽIVAČKIH REZULTATA U PRAKSU - SLUČAJ ISTARSKOG MLADOG KRUMPIRA

Anita Silvana Ilak Peršurić[1]

Original scientific paper

Summary

The paper presents data about the preferences and opinions of consumers toward young potatoes. The aim was to explain consumer habits with the goal of creating a new market brand of young potatoes produced in Istria. Using a questionnaire, we interviewed consumers on the green markets and in shops. The research sample contained 679 potato consumers throughout Croatia.
Two thirds of respondents were females, and most were under 55 years of age, with secondary or university education, with monthly income from 700 to 2000 euro, living in 4 member households. Their purchasing habits considered place, options in prices and quality and comparative features of branded and non-branded potatoes and organoleptic features (taste, smell, appearance).
Results showed that consumers buy mostly from green markets and in market chains, and less often buy directly from the producer. Potato organoleptic attributes (taste) and potato origin (domestic or local production) were crucial influences on buying behaviour. The main attributes important to consumption were sensory attributes of taste and smell. A brand created for these potatoes should confirm the value of the product (quality, domestic production, sensory attributes) and be more recognizable compared to potatoes sold in bulk. Research results have confirmed that the values of quality and security of the food and local production through a potato market label will be supported by Croatian consumers. The market label of the young potato would be most appealing to younger generations (under 25 years) more responsive to market labels; in smaller families (up to three family members), with income 800 to 1200 euro and slightly more by men, who trust market labels as a proof of quality.

Key words: *research results, transfer, consumers, potato.*

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Correspondence: Anita Silvana Ilak Peršurić, Department of Agricultural Economics and Rural development, Institute of Agriculture and Tourism, Tel: +385 52 408 329, Fax: +385 52 431 659, anita@iptpo.hr

Rezime

U radu su prikazani podaci o preferencijama i mišljenjima potrošača prema mladom krumpiru. Cilj je bio objasniti navike potrošača i koristiti ih u cilju stvaranja novog tržišnog brenda mladog krumpira proizvedenog u Istri. Koristeći anketni upitnik intervjuirali smo potrošače na zelenim tržnicama i u trgovinama. Istraživački uzorak sadržavao je 679 potrošača krumpira diljem Hrvatske.
Dvije trećine ispitanika bile su žene, većina je bila mlađa od 55 godina, sa srednjom ili visokom školom, i mjesečnim prihodima od 700 do 2000 eura, koje žive u kućanstvima sa 4 člana. Njihove navike u kupnji odnose se na mjesto kupnje, ponudu u cijeni i kvaliteti, usporedbi brendiranih i nebrendiranih proizvoda te organoleptičkim obilježjima (ukus, miris, izgled).
Podaci istraživanja pokazuju da potrošači imaju naviku kupnje na zelenim tržnicama i trgovačkim lancima, a rjeđe kod proizvođača. Organoleptički atributi krumpira (ukus) i porijeklo krumpira (domaća, lokalna proizvodnja) najjače su utjecali na ponašanje potrošača. Glavni atributi krumpira značajni za konzumaciju bili su senzorni atributi ukusa i mirisa. Brend kreiran za postojeći rinfuzni krumpir potvrđuje njegovu vrijednost kao proizvoda (kvaliteta, domaća proizvodnja, osjetilni atributi) i biti će prepoznatljiviji u usporedbi s krumpirom koji se prodaje u rasutom (rinfuza) stanju. Rezultati istraživanja potvrđuju važnost vrijednosti kvalitete i sigurnosti hrane te lokalnost proizvodnje za hrvatske potrošače te podršku novog brenda. Trgovačka oznaka mladog krumpira biti će jače podržana u segmentu mlađe generacije (ispod 25 godina) koja je pod jačim utjecajem tržnih marki; u manjim obiteljima (do tri člana obitelji) s prihodom od 800 do 1200 eura, i nešto više od muškaraca koji vjeruju da je tržišne oznake dokaz kvalitete.

Ključne riječi: *rezultati istraživanja, prijenos, potrošači, krumpir.*

INTRODUCTION

Consumption of food is a basic need; therefore it is a necessity highly ranked comparing to other needs, and satisfied first among other human needs (Maslovljev acc. to Foxall *et al*., 2007). The patterns of consumption regarding all food items are predominantly shaped in the primary social surroundings of the family. Through life events and experiences, consumption patterns can change towards to liking or disliking certain foods. Shaping the emotional experience toward food occurs in early childhood when “the mothers’ face imitates her child’s expression”, interpreting, reinforcing or shaping its gestures according to the cultural characteristics of the group to which they both belong (Ferrarini *et al*., 2010). Researchers measure such patterns through attitudes collected in surveys throughout questionnaires. Attitudes toward food can be measured through certain attitudes to attributes like taste, value for money and health benefits (Barrena and Sanchez, 2010; Ilak Peršurić *et al*., 2016). According to a consumer study by Fearne (1992), diet and health are the key attitudinal factors affecting potato demand. Habits and diet imply how much and how potato is consumed. In the Britian case (Gibson and Francis, 2015), the potato is considered as a healthy, low calorie food, rich in nutrients, consumed on 90g/day average. The most popular form of preparation was boiling, while the segment of children between ages 11-18 preferred potato as fried chips.

Very little data are available from the Croatian market research about consumption of food or how consumers verify certain attributes of food, like taste and product price (Kovačić *et al*., 2005). A survey by the State Statistical Office (www.novilist.hr) found that Croatian consumers consume 40 kg of potato annually. Similar findings were presented by Lupin and Rodrigez (2012) in which an average Argentinian consumer also consumed about 40 kg of potato annually.

Consumers usually choose a convenient place for purchase of food. According to Rodrigez *et al*. (2009) the consumer decides to buy at a certain place and then decides on the frequency of attendance. In the case of Italian and German consumers, potato was bought commonly once a month (Fitzsimmons *et al*., 2015).

At present, most of potatoes purchased and consumed on Croatian market are from an unrecognized source (producer) with no label and no guaranty of quality. Therefore, during this market label project "Istria young potato" financed by the Croatian Ministry of Agriculture we have tried to establish a new market label that would diminish stated market flaws. Our point was to prove that a labelled potato would become a more appealing market product comparing to bulk potatoes. Likewise, the American findings of Loureiro and Hine (2001) showed that the demand for a value added product would respond to a rise in market demand. In the case of Colorado potato, they showed that local origin was the most important feature and consumers were willing to pay higher prices (locality was even more important than the features of non-GMO and organic label). Possible enhancement of the volume of consumption was mentioned by Kolasa and Marks (1994), indeed more potato would be consumed if the label contained nutritional "content" and described positive nutritional benefits.

Marketing theories usually comprise all activities toward defining a marketing strategy for a product. Thus market research often begins with the consumers' needs (Ilak Peršurić and Težak, 2009). In the literature we can find measurements of potato attributes through mechanical, analytical and sensory methods (Povlsen *et al*., 2003). Findings relevant to our survey are sensory features of sight (colour), smell, taste (flavour) and touch (shape, peel). According to Lupin and Rodrigez (2012), potato attributes are perceived as a reflection of quality. In their case, Argentinian consumers preferred washed potato, middle sized and with brown colour peel, but could not differentiate between potato varieties.

In this paper we describe the attitudes of consumers toward attributes of the young potato, preferences about trade-marks and buying intentions toward the young potato in general and the future new trade-mark "Young Istria potato".

MATERIALS AND METHODS

Data presented in this paper was drawn from the field research on the project "Young Istria potato" financed by the Ministry of Agriculture, Forestry and Fishery of Republic Croatia. The young potato is according to State officials (www.nn.hr), a product put on markets for human consumption until 1st August. It is harvested before full ripeness and features a delicate, easily removable skin, healthy, whole, sturdy, and without scares, deformations, water core, scabs or diseases.

The sample of research consisted of consumers at green markets and retail stores throughout Croatia (namely Zagreb, Rijeka, Split, Pula, and Osijek). A total of 679 participants were selected in random order from consumers who were at the specified green market and retail stores on the days of research. The face-to-face survey was conducted using a questionnaire.

The questionnaire consisted of 34 questions organized in logical groups. Groups of questions referred to consumer behaviour when purchasing young potatoes, attitudes towards potato as a product, preferences for trade-marks, opinions about the needs for the new trade mark Istria young potato and potato attributes important for consumption. Most questions were close-ended with nominal or Likert scale responses (scale 1 to 5 where 5 was very important and 1 not at all important). The attributes of potatoes as a criterion for purchasing young potato and attributes of potato consumption were measured on a Likert scale with 1 indicating no importance at all and 5 meaning very important.

Data processing was done through standard statistical procedures (univariate, bivariate-correlations analysis).

RESULTS AND DISCUSSION

Sample description

The sample was gender uneven, as 71% respondents in the sample were women and 29% were men. We can speculate that these ranges are the consequence of the fact that more women than men shop groceries for the family, whereas the segmentation of consumers often shows that women tend to shop more often at green markets (as shown by Ilak Peršurić and Težak, 2009). Similar findings were reported in Europe by Fitzsimmons *et al.* (1998) in Italian (87%) and Germany (54%) cases where the samples were also predominantly female. In Argentina women were also the majority of the sample with 79% of the sample (Lupin and Rodrigez, 2012).

In our sample, most respondents had secondary (52.7%) and tertiary education (41.4%) while a small percentage had only an elementary education (5.9%).

Of the respondents 20.9% had income up to 3000 Kuna, 36.9% had 3 to 6 thousand Kuna, 21.7% had 6 to 9 thousand Kuna, 10.3% had 9-12 thousand Kuna and 10% respondents had more than 12 thousand Kuna (1 euro equals 7.5 Kuna, respectively, 400, 800, 1200, 1600 euro).

Regarding the household size, 8.7% had six family members, 20.3% had five, 33.8% had four, 20.1% had three, 10.8 had two family members and 6.3% were living in a single member household.
In terms of age 25.5% were aged between 16-24 years, 18% were 25-34, 25.5% were 35-44, 19.4% were 45-54, 8% were 55-65 and 3.4%were over 65 years of age.

Survey description of Purchase behaviour

We have tried to identify the purchase behaviour of the sample using the following set of questions: if they buy potatoes by themselves or if somebody else in the household does, their favourite and most frequent place of purchase, their consistency of purchase at stated place of purchase and reasons for frequenting the stated place of purchase (even if their favourite potato is not on sale).
The majority of consumers (45.7%) bought and consumed potatoes, while 40.7% consumed potatoes but someone else in the household purchased them.
Most consumers preferred large retail shops (39.4%) and green markets (38.4%) for the purchase of young potatoes. Less than 10% preferred other places of purchase like small shops (9.6%), buying from the farmer on a farm (9.4%) or other options (3.2%).
Reasons for choosing the particular place and consistency of purchase of young potatoes were explained by personal preferences toward that particular place (33.6%), wide range of offerings (29.2%), highest quality of products (17.6%), lowest price (11.2%) and other reasons (8.4%).
For the particular place of purchase 66.8% of respondents stated they had options for choosing several types of young potato (e.g. white, yellow, red and different varieties like Jearla, Red Scarlett, Adora). The rest (33.2%) stated they had no options at their place of purchase.
Most consumers (67.3%) were trusting their favourite place of purchase. If the young potato did not meet the desired criteria (size, appearance, price, package) or was of low quality (damaged, with scratches, with diseases), about 17.1% would buy on the same place at some other occasion, while only 16.1% would change the usual place of purchase.

Attributes of young potato and consumption behaviour

In the process of purchase the consumers evaluate a product according to their values, preferences, and habits before reaching a final decision regarding buying a product. Potato as a product has features of a low involvement product, meaning it has a low price and therefore consumers invest little time and effort into the purchase process.
Therefore for the field research we selected several concrete and abstract attributes that consumers of potatoes could easily evaluate. These attributes were ones on which consumers could quickly express opinions.
Extrinsic attributes were price, outlook (outward appearance), size, Croatian product and package (Table 1.), while intrinsic attributes were taste, smell, consistency and preparation (Table 2.).

The first attribute important to consumers was taste. Since the potato is a product consumed in various forms (chips, backed, boiled, puree etc.) sensorial characteristic were given priority. This feature was important or very important to almost 90% of respondents and thus was important information to producers who wish to grow varieties of potato that fit consumers' preferences.
The second attribute was geographical origin. About 77% of respondents stated that it was important or very important to have a Croatian product (that the potato was grown by Croatian farmers on Croatian land). This attribute is significant to farmers showing that Croatian consumers value the Croatian potato far more than imported ones. In addition it showed recognition of the value of the product in the eyes of the consumers.
Price being the third ranked attribute showed that consumers were concerned about cost, so farmers and retail stores should remain with the current pricing structure. We can expect that only a portion of the population might pay higher or premium prices for a new label of Istria young potato. When asked about their willingness to pay premium prices for a market branded potato consumers would only approve up to 20% increase of price.

Table 1. How important are the following potato attributes for purchase?

Attribute	Rank	Not at all (%)	Irrelevant (%)	Neither important or unimportant (%)	Important (%)	Very important (%)
Taste that I like	1.	1.0	2.3	10.4	45.6	40.7
Potato produced in Croatia	2.	3.5	4.2	11.9	32.3	48.1
Price	3.	0.3	4.6	18.2	39.4	37.5
Appearance	4.	2.6	10.1	17.5	53.9	15.9
Easy preparation	5.	3.3	13.1	23.9	42.0	17.7
Bulk potato	6.	5.2	16.1	28.9	28.9	21.0
Size of potato	7.	6.2	13.6	34.1	34.7	0.6
Packaged potato	8.	17.0	24.5	32.7	16.3	9.5

Source: market research, N = 679, authors' calculations.

The outward appearance of a young potato should show full ripeness, a delicate easily removable skin, healthy and sturdy outlook, without scares, deformations, water core, scabs or diseases. This attribute came in fourth place and was either important or very important for about 70% of consumers.
Easy preparation was important or very important for approximately 60% of consumers. Attributes ranked from sixth to last place were of less concern to consumers (important or very important only for one third of respondents). The fact that packaging was of least concern to consumers was valuable information for farmers and retail (packaging is an additional cost for farmers, while making transport and storage easier).

In the process of consumption, consumers rely on sensorial aspects of a product. Thus we have chosen several descriptive attributes and focused on smell, taste, consistency and preparation.

More than half of consumers (55.2%) stated that they recognize the difference between varieties of potato (yellow, white, red), while 30.3% see no difference (the remainder were unable to either confirm or dispute). The ones who recognize differences in varieties could also recognize differences according to geographical origin (20.2% potatoes produced in Lika, 18.9% Istria, 20.2 Dalmatia, 14.7% Međimurje and 11.9% Slavonija and Baranja).

Finally the main attributes of consumption were ranked. Taste was first ranked as important or very important to almost 90% of consumers. The other three attributes were ranked almost equally: smell was second ranked with 75% importance, preparation 73% and consistency in fourth place at 73.3%.

Table 2. How important are the following potato attributes for consumption?

Importance	Rank	Not at all (%)	Irrelevant (%)	Neither important or unimportant (%)	Important (%)	Very important (%)
Taste	1.	-	1.3	10.6	42.6	45.3
Smell	2.	1.4	3.7	18.6	40.8	35.5
Consistency	3.	1.4	3.5	21.7	41.6	31.7
Preparation	4.	1.9	6.0	18.5	47.0	26.6

Source: market research, N = 679, authors' calculations.

Since the purchase and consumption behaviour of the consumers was consistent and revealed the attributes needed for potatoes as a product, we examined which socio-economic features of consumers had an impact on purchase behaviour and implications of origin and label on consumers' choices (Table 3). Through bivariate analysis we have measured the strength of statistical correlations (¨˙Significant at p=0.001; ¨ significant at p=0.05, significant at p $\leq$0.05) between independent (gender, age, income) and dependent variables (purchase habits, importance of potato attributes and market labels).

In regard to age, consumers ages 35-44 were most involved in buying and purchasing potatoes. Other age groups, such as up to age 24 and over 44 were most frequently buying (less in consuming), while the group aged 25-34 consumed the most and bought the least.

For the age group most involved in buying and consuming potato (age 35-44) simple and easy preparation were most important. We can speculate that time saving aspects like simple and easy preparation and lower price were more important to women with four member families (working women) who lacked time and money (therefore buying in bulk).

The youngest (≤ 25) age group was less concerned with price and place more value on the packaging of potatoes. This age group of consumers (≤ 25) was most focused on appearance, size and taste of potato. The youngest consumers were less likely to buy potato in bulk. They preferred market labels because they considered labels as assurance of product quality. The youngest (≤ 25) age group was also most supportive to imported potato because it was packaged with a label (again connected to their preferences to labels and packaged product and outlook of potato). This age group was also most responsive to advertising and marketing promotion activities.

The oldest age group (≥ 55) had the least interest in market labels and were less demanding upon appearance and size of potato. About a third (29.7%) of consumers didn't want a market label because they were suspicious about the guaranteed quality and the price which is usually higher for branded products. In particular, women and those older than 55 were more concerned with the price of branded products.

Women tended to buy and consume more potatoes then men (about 90% women were frequent buyers in comparison to 79.1% of men), and consumed them more often (at least once weekly) then men (even in cases where someone else in the family made the purchase). Women tended to buy potatoes more often in retail chains, but men tend to buy them at green markets and directly from producers.

In general terms in our sample 35.2% of respondents had a consistent pattern of buying behaviour. Men's patterns were more consistent than those of women (always buying at the same place, even if the assortment, quality or price were not ideal).

Consumers were very positive about the market labels of food products in general (56.8% prefer labelled products) because they see it as a proof of quality. Men appreciated market labels more than women, felt more strongly that label is a guaranty of quality. Women focused more on quality, and were not convinced that a market label would guaranty quality.

Although not mentioned in tables, family size had a statistically significant influence on buying and consumption behaviour as did age, gender and income. We have noticed some relations that should be noted: the majority of families had four members, and these families were the most frequent purchasers and consumers of potato. In families with five and more members the frequency of purchase in retail shops was higher than for other sizes of families. Domestic production and potato labelled were most important to three and five member families.

Table 3. The effects of socioeconomic features of respondents on potato purchase habits

	Independent Variables								
	Gender			**Age**			**Income**		
Dependent Variables	**X^2**	**dF**	**Cc**	**X^2**	**dF**	**Cc**	**X^2**	**dF**	**Cc**
Do you buy young potato?	28.2	9	0.198	40.3	21	0.235	74.7	18	0.312
Where do you buy young potato?	16.3	18	0.152	45.1	42	0.248	92.9	18	0.312
Why do you buy at that certain place?	34.2	21	0.217	47.2	49	0.353	115.7	42	0.379
Are there possibilities of choice?	70.1	9	0.710	16.1	12	0.711	76.0	18	0,725
Would you buy potato at that certain place although no appropriate potato is available?	69.8	12	0.709	15.3	18	0.710	75.9	24	0.724
Potato attributes important for purchase	70.5	21	0.711	23.4	24	0.266	24.3	25	0.232
Way of preparation / consumption of potato	75.0	51	0.721	18.7	24	0.764	34.7	20	0.324
Known and recognizable place of production?	69.6	9	0.709	13.1	16	0.402	80.8	55	0.331
Would you prefer domestic over imported potato?	69.6	9	0.708	11.7	12	0.134	66.4	15	0.308
Do you recognize market labels?	71.5	12	0.713	36.8	36	0.227	21.5	10	0.171
Do you prefer market labels?	71.3	9	0.712	94.8	30	0.350	12.8	25	0.405
Would you buy labelled Istria Young potato?	71.1	18	0.712	27.1	12	0.196	33.5	10	0.220

	Gender			**Age**			**Income**		
Dependent Variables	**M**	**SD**	**F**	**M**	**SD**	**F**	**M**	**SD**	**F**
Do you buy young potato?	2.19···	1.22	8.41·	2.19	1.15	0.60	2.19	1.15	20.38···
Where do you buy young potato?	0.97	1.25	3.48	0.97	1.25	1.14	0.97	1.25	9.14···
Why do you buy at that certain place?	1.34	1.75	7.42	1.34	1.72	0.12	1.34	1.71	18.77···
Are there possibilities of choice?	0.63···	0.73	13.42···	0.63	0.73···	5.00·	0.63	0.73	1.32·
Would you buy potato that day and place although your favourite kind is not sold?	1.00···	1.16	23.92···	1.16	0.04···	2.27	1.00	1.16	8.39···
Known and recognizable place of production?	0.70···	0.8	43.72···	0.83	0.03···	11.53···	0.70	0.83	0.003···

Would you prefer domestic over imported potato?	0.67···	0.94	86.70···	0.67	0.95···	27.47···	0.67	0.94	5.22
Do you recognize market labels?	1.01···	0.67	49.18···	1.01	0.67···	82.67···	1.01	0.67	80.91···
Do you prefer market labels?	1.21	0.85	56.73···	1.23	0.84···	50.92···	1.23	0.85	55.24···
Would you buy labelled Istria Young potato?	1.44···	0.55	98.97···	1.16	0.77···	38.85···	1.54	1.44	32.91···
Why would you buy labelled Istria Young potato?	1.55···	0.59	25.10···	0.68	1.55···	67.33···	0.68	1.55	36.78···

Source: market research, N = 679, authors' calculations.

Income also influences the percentage of potato consumption, the higher the income the more consumption occurs (above 800 euro). With the rise of income the percentage of persons which consume but do not buy potatoes rises. We can speculate that with higher incomes the working hours of these consumers are longer and therefore they have little time for grocery shopping, leaving someone else in the family to make purchases. As income grew (above 1200 euro) potatoes were more frequently bought at retail chains and less in green markets. The loyalty to a certain place of purchase (even in the case of no appropriate potato being available) was the strongest at incomes from 800 to 1200 euro. Potato attributes of domestic production, appearance, easy preparation and potato packaging was also most important to the income group 800 to 1200 euro.

After the examination of purchase behaviour and potato attributes, we asked with questions about a new market label "Istria young potato" for existing bulk potatoes (already on the market). Through a set of questions about market labels in general, preferences to local, domestic or foreign production and prospects of young potato produced in Istria we attempted to identify important factors for the new market label.

The majority of men (75.5%) and women (78.4%) expressed their interest in a new product named "Istria young potato". Most of the sample (about 71.2%) were positive toward the new label, stating that the market label will be a warranty of local quality (Istria) and that the market label would provide exact desired attributes like taste, smell, consistency and preparation (43.8%). Men had slightly more positive attitudes toward the new label because of known and recognizable place of production in comparison to women wo were more skeptical that the label would be a warranty of quality and local production.

DISCUSSION AND CONCLUSION

This marketing and technology research project financed by the Croatian Ministry of Agriculture had several aims: to examine the behaviour of consumers to young potato as a product, to validate consumers' attitudes and opinions about potato attributes and to use this information in creating a new market label.

For producers the research data and findings about the consumers and the market were a valuable source of information and were used for the new market label "Iskro" the Istrian young potato currently sold on the market in Croatia, in retail and at green markets.

Our sample consisted of 679 potato consumers from markets in four Croatian cities, Zagreb, Rijeka, Split, Pula and Osijek. The research tool was a questionnaire.

The data showed that Croatian consumers had major preferences for potatoes purchased in retail chains and at green markets. Respondents had a convenient place of purchase and were consistent in visiting it for potato purchase. Similar to the findings of Rodrigez *et al.* (2009) our sample indicated that they choose a place of purchase and would not change it for another place.

In our sample the respondents with income above 800 euro were most consistent in their preferences for the place of purchase of potato tending to choose retail chains. Additionally these respondents were less frequent buyers because lack of time (somebody else in the family purchased potatoes).

Even in cases when their favourite potato was not available in the shop or green market the consumers would still buy it at the chosen place (consistently across age, gender and income).

The decision process of buying young potatoes was built upon sensorial characteristics, with preferences for taste and smell ranked highest, similar to findings of Povlsen *et al.* (2003). Since young potato is a simple product with low involvement (different from high-involvement products like wine, olive oil or organic products) producers should market it with a market label and attributes of geographical origin, quality, flavour and value. Croatian origin was also ranked highly, while package was considered least important.

Method of preparation (cooked, fried, mashed) was related to gender and age: younger respondents preferred fried, while older preferred mashed potatoes (similar to Gibson and Francis, 2015).

In general market labels were recognized by our respondents as a sign of recognition of Croatian origin and quality. For younger respondents (under 25 years of age), market labels were most appealing and they endorsed the new market label Istrian young potato more than older respondents. The respondents older than 55 were least interested in market labels, including the new market label Istrian young potato.

The price of the potato with the new market label Istrian young potato could be as much as 20% higher price than bulk potato.

Although men and younger respondents were less wary about the label as warranty of quality than women and older respondents, women were the most frequent buyers. Thus marketing should prioritize targeting women.

The label Istrian young potato would be a guarantee of Istria quality, recognizable place of production (Istria), unique effect of terroir (red soil, Mediterranean climate, seaside influence) and sensorial characteristics (smell and taste).

Farmers should remain with their stands at the green markets as a place of direct sales with the possibility of direct contact with consumers (and consumers' preferred place of purchase) and in the meantime enter large retail chains with their new labelled product at a higher price point.
Advertising should put emphasis on excellent taste and smell originating from the red mineral soil and influence of the Mediterranean sun while the label suggests that the product is produced locally in the Croatia and is ecologically friendly (requires less use of non-renewable resources for transport).

REFERENCES

Barrena, R., Sanchez, M. (2010): Frequency of consumption and changing determinants of purchase decision: from attributes to values in the organic food market. Spanish Journal of Agricultural Research. Vol. 8, No 2, pp 251-272.

BrunsǾ, K., Grunert, K. G. (2007): Consumer attitude measures and food product development. In H.McFie (Ed.) Consumer-led food product development. CRC Press, New York, USA. pp:197-222.

Fearne, A. (1992): The Great British Potato: A Study of Consumer Demand, Attitudes and Perceptions". British Food Journal. Vol 94, No 6; pp 22 – 28.

Ferrarini, R., Carbognin, C., Casarotti, E. M., Nencini, A., Meneghini A. M. (2010): The emotional response to wine consumption. Food Quality and Preference No 21; pp 720-725.

Fitzsimmons, J. A., Colantuoni, F., Cicia, G., Del Giudice, T. (2015): Human values and Consumers Preferences for Extrinsic Credence attributes in the Italian and German markets for new potatoes, at www.agroeconreserch.umn.edu/bitstream/202745/2/240

Foxall, G. R., Goldsmith, R. E., Brown, S. (2007): Psihologija potrošnje u marketingu (Consumer Psychology for Marketing). Naklada Slap. Jastrebarsko. Croatia.

Gibson, S. A., Francis, L. (2015): An analysis of potato consumption habits and diet quality among adults and children in the UK. British Nutrition Foundation. Nutrition Bulletin No 40; pp 177-186.

Ilak Peršurić, A. S., Težak, A. (2009): The influence of socio-demographic characteristics of tourists on their interest for organic food in Istria, Croatia. Agricultural Economics Vol. 10, No 6; pp 296-305.

Ilak Peršurić, A. S., Težak Damijanić, A., Saftić D. (2016): Winery and wine exhibition visitors' characteristics in the context of wine tourism development. New Medit No 4; pp 82-89.

Kolasa, K., Marks, B. (1994): Nutrition public relations: dietary promotion of the potato. Nutrition today January/February: pp. 30-36.

Kotler, P. (2001): Upravljanje marketingom: analiza, planiranje, primjena i kontrola. (Marketing management). Mate d.o.o., Zagreb.

Kovačić, D., Mesić, Ž., Pavičić, A. (2009): Analysis of organic food demand in Zagreb market. Proceedings 44.Croatian and 4th International symposium on agriculture: pp 213-217. Croatia.

Loureiro, M. L., Hine, S. (2001): Discovering niche markets: A comparison of Consumers' willingness to Pay for A local (Colorado-grown), Organic, and GMO–free product. http://www.researchgate.net/publication /23504489

Lupin, B., Rodrigez, E. M. M. (2012): Quality attributes and socio-demographic factors affecting channel choices when buying fresh potatoes in Argentina. Poster prepared for International Association of agricultural economists, Brazil.

Moskowitz H. R., Krieger, B. (1993): What sensory characteristics drive product quality? An assessment of individual differences. Journal of Sensory Studies Vol 8; pp 271-282.

Povlsen, V. T., Rinnan, A., Van den Berg, F., Andersen, H. J., Thybo, A. K. (2003): Direct decomposition of NMR relaxation profiles and prediction of sensory attributes of potato samples. Lebensmittel - Wissenschaft und Technologie, Vol 36, No 4; pp 423-432.

Rodrigez, D. M. C, Caceres Hernandez, J., Guirao Perez, G., Cano Fernandez, V. J. (2009): Individual profiles and wine consumption patterns in Tenerife. A Multinominal Logit Model. Spanish Journal of Agricultural research Vol 7, No 4; pp 759-769.

http://www.novilist.hr/Vijesti/Gospodarstvo/Hrvati-godisnje-pojedu-40-kilograma-krumpira-i-sve-to-zaliju-sa-18-litara-piva (State Statistic Office survey) accessed at 24. July 2015.

http://narodne-novine.nn.hr/ accessed at 24. July 2015.

INOVATIVNI RAZVOJ NOVIH VINSKIH CESTA UPORABOM NALAZA ISTRAŽIVANJA PREFERENCIJA PREMA VINU*

INNOVATIVE DEVELOPMENT OF NEW WINE ROADS USING RESEARCH RESULTS ON PREFERENCES TO WINE

Anita Silvana Ilak Peršurić[1]

Originalni znanstveni rad

Rezime

Stvaranje novih i inovativnih tipova ponude koji su namijenjeni segmentima turističke potražnje u turističkoj destinaciji uobičajeno je prepušteno turističkim subjektima ili lokalnim turističkim zajednicama. Uporaba institucionalnih kapaciteta fakulteta i znanstvenih instituta u kreiranju novih inovativnih programa za turizam manje je uobičajen put. Stoga je provedeno istraživanje s naglaskom na autohtona vina i autohtone vinske sorte te sukladno stavovima potrošača u istraživanju kreirati nove tematske turističke itenerere i vinske rute. Osnovna hipoteza bila je da postoje razlike u populaciji domaćih i inozemnih potrošača vina, temeljem koje smo istražili dvije skupine potrošača, lokalno stanovništvo i turiste. Slijedom toga druga hipoteza bila je da postoje razlike u preferencijama prema atributima vina i navikama u konzumaciji vina. Nalazi istraživanja pokazuju značajne razlike u preferencijama prema atributima vina dviju skupina (lokalno stanovništvo više cijeni atribute od turista). Unutar dviju skupina zamijećene su razlike u konzumaciji u odnosu na spol, dob i prihode.

Ključne riječi: *Vinske ceste, potrošači, atributi vina, Malvazija Istarska.*

Summary

The creation of new and innovative types of tourism offers intended for segments of tourist demand in a tourist destination is usually left to tourist entities or local tourist communities. Using the institutional capacity of faculties and scientific institutes to create new innovative tourism programs is less common. The research was set to give emphasis to indigenous wines and autochthonous wine varieties and according to consumer attitudes in research to create new thematic tourist itineraries and wine routes. Our main hypothesis was that differences between the population of domestic and foreign wine consumers exists and therefore we have examined two samples of consumers, domestic population and tourists. Further, our second hypothesis was that

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Correspondence: Anita Silvana Ilak Peršurić, Department of Agricultural Economics and Rural development, Institute of Agriculture and Tourism, Tel: +385 52 408 329, Fax: +385 52 431 659, anita@iptpo.hr

there are differences in preferences toward wine attributes and wine consumption habits. Research results showed important differences in preferences towards wine attributes between two samples of consumers (local population and tourists). Inside both of the samples differences in consumption were evident regarding gender, age and income.

Key words: *Wine roads, consumers, wine attributes, Malvazija Istarska.*

UVOD

U najopćenitijem pogledu turizam podrazumijeva boravak izvan mjesta uobičajenog boravka koji je potaknut ili motiviran izvjesnim faktorima motivacije tzv. intrinzičnim ili ekstrinzičnim faktorima. U literaturi se u tom smislu spominju "push" i "pull" faktori tj. faktori unutarnjih motiva osobe "guranja" i "privlačenja" prema nekoj turističkoj destinaciji ili sadržaju. Prema Dann-u (1977, 1981) faktori "guranja" su bijeg od svakidašnjice, relaksacija, prestiž, istraživanje, poboljšanje odosa u obitelji i olakšavanje međuljudskih odnosa. Faktori "privlačenja" su novosti i znanja o destinaciji ili atraktivnosti i sadržaji turističke ponude.
Boravak u destinaciji koji je potaknut vinom kao proizvodom u destinaciji te glavnim motivom koji sačinjavaju posjete vinarijama, vinskim festivalima i drugim događajima vezanim uz vino u nekoj destinaciji, nazivamo vinskim turizmom (Getz, 2000; Hall i Macionis, 1998; Hojman i Hunter-Jones, 2012). Vinski turizam može biti i dio drugih vidova turizma kao npr. ruralnog, seoskog, obalnog turizma i sl. (Yuan *et al.*, 2005).
Vino kao proizvod privlači potrošača svojim atributima, kako ekstrinzičnim tako i intrinzičnim kvalitetama. Od ekstrinzičnih navode se porijeklo i vrsta grožđa, pakiranje tj. ambalaža, cijena, dok su intrinzični atributi vrijednost koju vino daje za određeni iznos novca (vrijednost za kupca), reputacija, mišljenja stručnjaka i ocjenjivača vina te senzorske kvalitete.
Najčešće su istraživane poveznice između cijene vina i zadovoljstva potrošača (Ampuro i Villa 2006; Duarte 2010; Chrea *et al.*, 2011) te vrijednost za potrošača (Cardebat i Fiquet, 2009; Outreville 2011.) tj. koju vrijednost vinu pridaju potrošači, a može biti neovisna o tržišnoj cijeni i predstavljati očekivanja i vrednovanja potrošača koja se možebitno i razlikuju od stavova ocjenjivača ili javnosti. Neki atributi vrednovani od vinskih eksperata mogu pozitivno utjecati na potrošača i navesti ga na kupnju. Važnost senzornih atributa poput boje, arome, ukusa, aroma zrenja i starenja mogu značajno utjecati na kupnju i konzumaciju vina (Chrea *et al.*, 2011).
Vinski turisti kao dionici vinskog turizma motivirani su posjetiti destinaciju jer je dio neke vinske regije i sadrži vinske ceste sa proizvođačima vina. Namjera im je iskusiti destinaciju kao ruralnu idilu (Getz 2000; Getz i Brown 2006), vinograde kao dio prirodnog krajolika (Tempesta *et al.*, 2010), vino kao dio turističke potrošnje (Hall 1998; Sparks 2007), vino kao motiv dolaska (Bruwer i Li 2001; Crompton i

McKay 1997) te posjetiti vinarije na vinskim cestama i vinske sajmove u destinaciji (Ilak Peršurić i Červar 2012).
Vinske turističke ceste prema Zakonu o turističkoj djelatnosti (NN 8/96) jesu „organizirani skup turističkih atrakcija, turističke infrastrukture i osoba na kojima se stanovnici bave vinogradarstvom te proizvodnjom i prodajom vina…“(www.nn.hr).

Slijedom potrebe za novim turističkim sadržajima u Istri, vezanim uz vino, a koji nadograđuju postojećih sedam vinskih cesta u Istri izvršili smo istraživanje o zahtjevima potrošača prema vinu (atributi vina) te o potrebama za novim turističkim sadržajima i povezali ih kroz postojeće nalaze prethodnih znanstvenih projekata (Ilak Peršurić *et al.,* 2016; Težak Damijanić *et al.*, 2015).

MATERIJALI I METODE

Tijekom trajanja projekta „Putevima Malvazije Istarske - Malvazija TourIstra“ (2014 – 2015) financiranog putem programa prekogranične suradnje IPA Slovenia – Hrvatska istražili smo postojeću proizvodnju vina, tipove Malvazija, kemijske i organoleptičke značajke te ponašanje potrošača u vinskim podrumima, na vinskim sajmovima te stavove i preferencije prema vinima i Malvaziji Istarskoj.
Osnovna hipoteza bila je da postoje dvije skupine potrošača vina u Istri, i to domaće stanovništvo i turisti. Pretpostavka je bila te dvije skupine imaju različit socio demografski i ekonomski profil i slijedom toga da se ponašanje tih dviju skupina razlikuje.
Dodatna hipoteza bila je da atributi vina nemaju isti učinak na domaće stanovništvo i turiste, te da će domaće stanovništvo iskazati viši stupanj preferencije prema vinu, dok smo za turiste pretpostavili da će biti više zainteresirani za sadržaje destinacije Istra.
Odabrana su dva uzorka istraživanja; posjetitelji vinskih podruma (turisti) te lokalno stanovništvo. Turisti su anketirani u 30 vinskih podruma na vinskim cestama Istre, a uzorak je bio 107 ispitanika, dok je lokalno stanovništvo anketirano putem web aplikacije te je uzorak sadržavao 120 ispitanika.
Za obje grupe anketni upitnik je sadržavao ista pitanja podijeljenih u četiri odrednice; 1) socio demografske odrednice, 2) motivi za konzumaciju vina, 3) preferencije prema vinu, 4) aspekti vinskih podruma i vinskog turizma.
Motivi za konzumaciju vina, preferencije prema vinu i aspekti vinskog podruma i turizma mjereni su Likertovom skalom, ocjenama od 1 do 5 (pri čemu je jedinica značila „uopće se ne slažem“ do petice koja je značila u potpunosti“ se slažem s navedenom tvrdnjom u pitanju).
Podaci su podvrgnuti standardnim tehnikama univariatne (srednje vrijednosti i postoci) i bivariatne analize (hi kvadrat test i koeficijenti korelacija varijabli).

REZULTATI I DISKUSIJA

Turisti, posjetitelji vinskih podruma u većini su bili muškarci (2/3), između 35 i 54 godina sa višim stupnjem obrazovanja (fakultet) bili su najčešće samozaposleni ili zaposlenici u tvrtkama, a svaki peti je bio na poziciji rukovoditelja ili direktor tvrtke, i u rangu najvišeg dohotka (iznad 4 tisuće eura mjesečno). Većina ih je bila iz Italije i Njemačke, i to su već tradicionalno najčešći posjetitelji Istre kao turističke destinacije. Manjina ih je bila iz Austrije, Nizozemske i Engleske.
Lokalno stanovništvo predstavljaju većinom žene u tridesetim godinama života, visokoobrazovane i sa srednjom ili nižom razinom primanja.
Između dviju skupina jasne su razlike u spolu gdje u uzorku lokalnog stanovništva prevladavaju žene, dok u uzorku turista prevladavaju muškarci.
Lokalno stanovništvo ima sličnu dobnu strukturu kao turisti, ali i značajno višu razinu obrazovanja u odnosu na turiste.
Turisti su po zanimanju češće bilo samozaposleni i menadžeri, dok je lokalno stanovništvo češće bilo zaposlenik u tvrtki, slijedom toga turisti su imali i značajno višu razinu dohotka.

Tablica 1. Socio demografski profil ispitanika

Socio demografski Indikatori		Lokalno Stanovništvo Postotak (%)	Vinski turisti Postotak (%)
Spol	Muški	33.9 %	63 %
	Žene	66.1 %	37 %
Dob	Do 24	12.9 %	12 %
	25-34	24.2 %	17 %
	35-44	27.4 %	36 %
	45-54	24.2 %	20 %
	55+	11.3 %	15 %
Obrazovanje	Osnovno	-	1 %
	Srednje	11.3 %	39 %
	Više i Visoko	88.7 %	60 %
Zemlja	Hrvatska	100 %	25 %
	Italija	-	16 %
	Njemačka	-	16 %
	Slovenija	-	6 %
	Belgija	-	4 %
	Nizozemska	-	4 %
	Rusija	-	4 %
	Ostalo	-	18 %

Izvor: autorsko istraživanje

Tablica 2. Ekonomski profil ispitanika

Ekonomski indikatori		Lokalno Stanovništvo Postotak (%)	Vinski turisti Postotak (%)
Mjesečni prihodi	Do 1.000 €	60.5 %	26 %
	1001-2000 €	29.5 %	29 %
	2001-3000 €	-	19 %
	3001-4000 €	-	6 %
	Više od 4000 €	-	20 %
Zaposlenje	Poduzetnik / vlastiti posao /samozaposlen	12.9 %	31 %
	Zaposlenik	62.9 %	32 %
	Menadžer	6.5 %	20 %
	Đak / student	12.9 %	10 %
	Umirovljen	1.6 %	4 %
	Ostalo	3.2 %	3 %

Izvor: autorsko istraživanje

PONAŠANJE ISPITANIKA U KONZUMACIJI I KUPNJI VINA

Utvrđivanje razlika u ponašanju potrošača vina i dokazivanje osnovne hipoteze proveli smo setom pitanja o potrošačkim navikama u odnosu na vino. Istražili smo koliko mjesečno lokalno stanovništvo i turisti troše za vino, na koji način (rinfuza, boc, buteljke), koje vino preferiraju (mlado ili odležalo) koji tip vina (suho, polusuho, poluslatko, slatko) i koji način odležavanja (inox, drvo).

Na pitanje o preferiranom stilu vina više od polovice ispitanika izjavila je da preferira atribut mladog svježeg vina. Jedna četvrtina preferira vino odležano u drvenim bačvama, dok manji udio potrošača preferira vino Malvazije odležano u inoxu ili u boci. Slatko, desertno vino najmanje je zanimljivo potrošačima.

Konzumacija vina odvijala se većinom u vlastitom kućanstvu, na taj način je 75% vinskih turista i 60.4% lokalnog stanovništva konzumiralo vino. Sljedeći omiljeni način konzumacije bio je izvan kuće, u ugostiteljskim objektima je 24.2% vinskih turista i 23% lokalnog stanovništva konzumiralo vino.

Osim navedenog lokalno stanovništvo je u odnosu na turiste pokazalo i veću varijabilnost u konzumaciji jer je u noćnim barovima i klubovima vino konzumiralo 11% ispitanika, u agroturističkim domaćinstvima 2.2% i na drugim mjestima 2.2%.

Većina lokalnog stanovništva je konzumiralo vino u bocama od 0.75 L (89% ispitanika), a manji dio u drugim tipovima pakiranja (6% u litrenim bocama i 5% u rinfuzi).

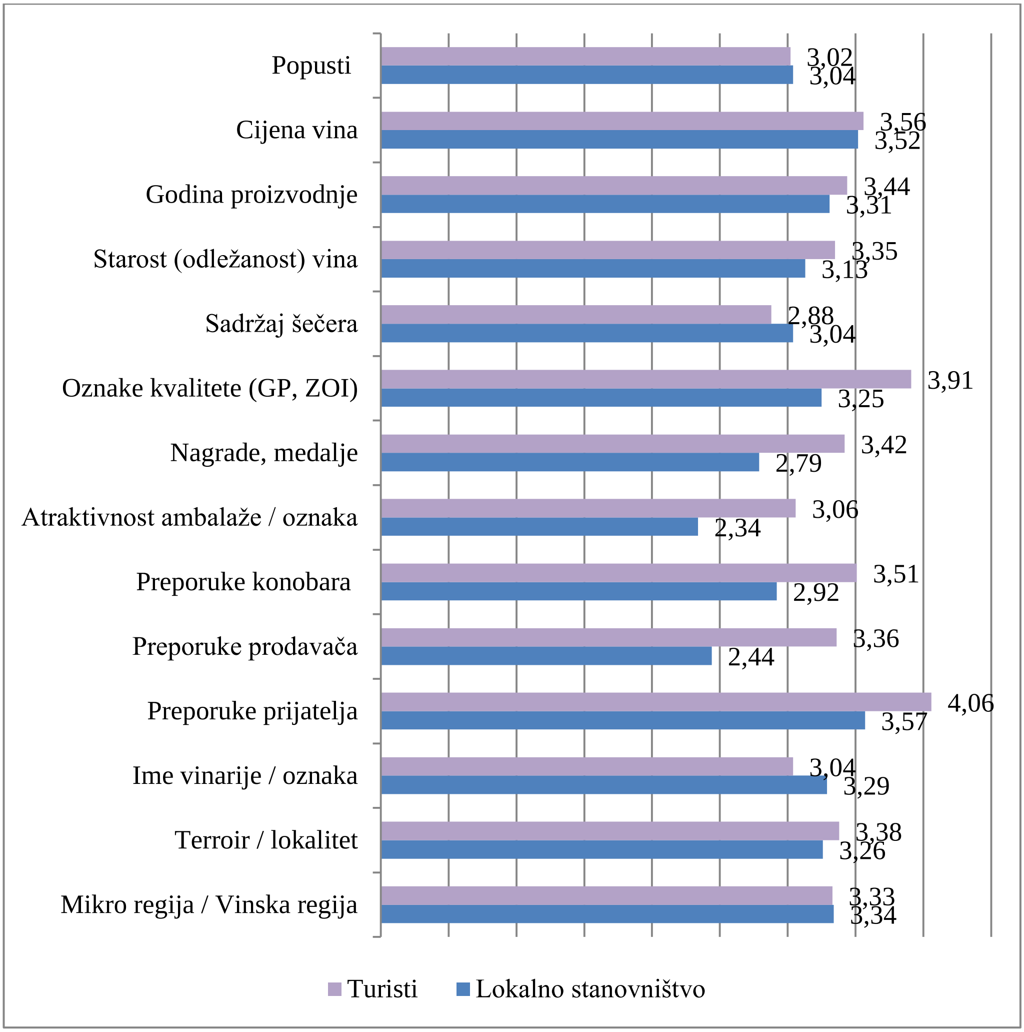
Lokalno stanovništvo je kupovalo vino većinom kod vinara (40%) ili u trgovačkim centrima (39%), a rjeđe u specijaliziranim trgovinama (enotekama, 20%) ili putem interneta (1%).

Potrošačke navike ispitanika lokalnog stanovništva odražavaju se i kroz raspoloživost mjesečnih prihoda, tako je većina lokalnog stanovništva trošilo do 20 eura mjesečno

za vino (66.2%), oko dvadeset posto je trošilo 21-35 eura, 19.5% između 36 i 55 eura i 5.2% više od 55 eura mjesečno.
Turisti su trošili veće svote mjesečno za vino, tako je 26% trošilo do 20 eura, 24% od 21 do 35 eura, zatim 34% od 36 do 55 eura i 16% više od 55 eura mjesečno.

PREFERENCIJE POTROŠAČA PREMA ATRIBUTIMA VINA

Radi utvrđivanja razlika u preferencijama prema vinu u dvjema skupinama potrošača vina (domaće stanovništvo i turisti) postavili smo set pitanja o vinu kao proizvodu sa svojim kvalitetama tj. „atributima“. Ispitanici su ocjenom jedan do pet iskazali ocjenu važnosti svakog pojedinog atributa te su rezultati preferencija prikazani na Graf. 1.
Atribute vina smo podijeli na ekstrinzične (pakiranje, cijena, popusti, vinska/ mikro regija) i intrinzične atribute (oznake kvalitete - geografsko porijeklo, IQ – oznaka Istarske kvalitete, terroir, nagrade, sadržaj šećera, godište, starost vina, preporuke prijatelja i prodajnog osoblja).
Intrinzični atribut preporuke prijatelja za neko vino bio je najviše ocijenjeni atribut vina (ocjena 4.06). Od atributa preporuka drugih osoba za vino važan je atribut preporuke prodajnog osoblja (konobari 3.51 i trgovci 3.36).
Lokalno stanovništvo davalo je više ocjene za intrinzične atribute u odnosu na vinske turiste (primjerice za preporuke prijatelja 4.06 naprema 3.57, preporuke konobara 3.51 naprema 2.92 i preporuke prodavača 3.36 naprema 2.44).



Graf 1. Atributi vina - ocjene važnosti

Izvor: autorsko istraživanje

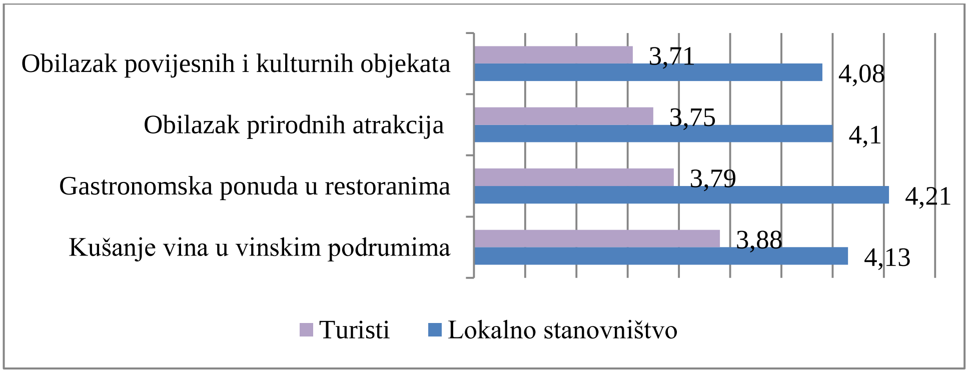
Od ostalih intrinzičnih atributa lokalno stanovništvo visoko je ocijenilo kvalitetu vina (3.91) i postignute medalje i nagrade (3.42).
Cijena vina bila je neznatno važnija od njegovog godišta (za 0.1 bod), ali manje važna od njegove kvalitete (za 0.4 boda manje).
Lokalno stanovništvo je pridavalo veću važnost i ostalim intrinzičnim atributima u odnosu na vinske turiste i za sve ekstrinzične atribute vina.

Malo veću ocjenu, neznatno višu ocjenu dali su turisti za atribute popusta u cijeni (0.1 više) te turisti su dali nešto višu ocjenu za naziv vinarije ili oznake vinarije (turisti 3.29 u odnosu na 3.04 za lokalno stanovništvo).

Navedene razlike možemo tumačiti nešto većom osjetljivošću turista na cijenu vina, bez obzira na njihovu relativno veću platežnu moć, te na važnost prepoznatljivosti vina i vinarije (a koja je vizualno prepoznatljiva kroz oznaku vinarije i vinske regije na ambalaži).

MOTIVI ZA DOLAZAK U TURISTIČKU DESTINACIJU I KONZUMACIJU VINA

Radi utvrđivanja važnosti koju vino kao proizvod ima kao dio ponude za turističku destinaciju Istra u slijedećem koraku istraživanja nastojali smo saznati koliko je vino važno kao motiv za dolazak u destinaciju kao atraktivni čimbenik, a koliko su važni ostali čimbenici ponude.
Ispitanici su ocjenjivali na skali od jedan do pet (pet najviša ocjena) koliko ih motivira obilazak atrakcija i gastronomska te vinska ponuda u destinaciju Istra. Naša pretpostavka bila je da će turisti bili više zainteresirani za sve aspekte ponude u destinaciji u odnosu na lokalno stanovništvo koje živi u destinaciji.
Iz nalaza istraživanja dviju skupina ispitanika (Graf 2.) vino se pokazalo važnim u dvjema najvažnijim motivima za dolazak u destinaciju i to u gastronomskoj ponudi i u kupnji vina u destinaciji. I lokalnom stanovništvu i turistima ova dva motiva vode u odnosu na druge prirodne i kulturne atraktivnosti destinacije.



Graf 2. Motivi za posjetu destinaciji Istra

Izvor: autorsko istraživanje

Međutim, suprotno očekivanome, lokalno stanovništvo bilo je motiviranije za obilazak prirodnih atrakcija i povijesnih i kulturnih objekata u odnosu na turiste i shodno tome je lokalno stanovništvo davalo više ocjene za parametre posjeta Istri.
U odnosu na nalaze istraživanja preferencija prema atributima te iskazanoj važnosti utjecaja ljudi iz najbližeg okružja ispitanika (prijatelji, poznanici, obitelj) i ljudi koji sudjeluju u konzumaciji i prezentaciji vina (konobari, ugostitelji, vinari) nalazimo da vinari i osoblje u vinarijama i ugostiteljskim objektima mogu značajno pospješiti prodaju i prezentaciju vina kao proizvoda i prema lokalnom stanovništvu i prema turistima. Također mogu značajno doprinijeti doživljaju vinske i turističke destinacije,

a vino kao njihov proizvod i dio destinacije značajno može doprinijeti ukupnoj ponudi i motiviranosti za posjetu Istri.
Radi dubljeg uvida u međuovisnost socio demografskih i ekonomskih obilježja ispitanika i njihovog ponašanja u kupnji vina i preferencijama prema atributima vina izvršena je bivarijatna analiza. Iz setova varijabli izdvojene su statistički najjače povezane čiji su rezultati prikazani u tablicama 3 i 4.

Tablica 3. Korelacije socio demografskih i ekonomskih obilježja lokalnog stanovništva i preferencija prema atributima vina i ponašanje u konzumaciji vina

	Lokalni - Dob			*Lokalni - Dohodak*			*Lokalni - Spol*		
	χ^2	dF	Cc	χ^2	dF	Cc	χ^2	dF	Cc
Konzumacija vina									
Mjesečna potrošnja	22.9	24	0.520	30.7	12	0.896	15.6	6	0.448
Suho vino	4.34	4	0.263	7.2	4	0.334	16.2	1	0.465
Mjesto konzumacije	29.5	16	0.578	20.7	16	0.513	6.2	4	0.309
Informiranost o vinskim podrumima									
Od prijatelja/poznanika	6.8	4	0.323	28.5	24	0.564	20.7	16	0.513
Atributi vina									
Mikro /vinska regija	12.3	16	0.424	16.1	16	0.476	2.8	4	0.219
Terroir / lokalitet	18.3	16	0.303	15.2	16	0.463	2.5	4	0.207
Atraktivnost oznake / imena	25.3	16	0.535	22.6	16	0.536	0.7	4	0.115
Preporuke prijatelja	27.6	16	0.568	14.1	16	0.446	4.2	4	0.260

Izvor: izračun autora

U ponašanju ispitanika (lokalnog stanovništva) prema uobičajenom mjestu potrošnje, a to je bilo u kućanstvu, uočene su razlike u ponašanju, pri čemu je najjači utjecaj imala dob, potom dohodak a najmanje spol. U tom smislu muškarci troše više novčanih sredstava na vino mjesečno od žena i oni preferiraju više suho vino dok dio žena preferira slatka ili poluslatka vina i troše mjesečno manje na vino (vezano uz njihova niža primanja).

Mlađe dobne skupine (do 35 godina) češće konzumiraju slatka i polusuha vina od ostalih i jače su pod utjecajem atraktivnosti ambalaže kad kupuju vino od starijih dobnih skupina. Mlađi potrošači (do 35 godine) su bili i pod jačim utjecajem preporuka prijatelja u odabiru vina u odnosu na starije.
Mjesečna potrošnja vina izražena novčano (u eurima) najviša je u dobnim skupinama od trideset pet do pedeset godina (u toj skupini je veći udio potrošnje iznad 30 eura mjesečno) dok je u višim dobnim (iznad 55) i mlađoj skupini niža potrošnja (ispod 24 godine starosti).
Dohodak se pokazao kao čimbenik utjecaja na odabir vina kroz utjecaj prijatelja i konobara i to najviše na dohodovnu skupinu do tisuću eura, a u skupini dohotka iznad

tisuću eura povećana je važnost atributa vina „terroir“ odnosno lokalitet proizvodnje vina, te mikroregija proizvodnje.
Ispitanici u dobi od 35 do 54 također su naglasili atribut „terroir“ odnosno lokalitet proizvodnje vina, te mikroregija proizvodnje.
Turisti u dobi 18 do 24 godine preferiraju slatka i poluslatka vina, a u dobi 25 do 34 preferiraju suha vina. U dobi od 25 do 45 turisti troše više mjesečno na vino u odnosu na ostale dobne skupine. Muškarci češće konzumiraju vino izvan kuće, i to u restoranima, gostionicama i barovima, a važniji im je atribut vina „terroir“ mikroregija i lokalitet u odnosu na žene.
Mlađi turisti, u dobi do 34 godine također češće konzumiraju vino izvan kuće, i češće su zainteresirani za tematske itenerere i organizirane posjete vinskim podrumima i vinarijama.
Muškarcima i turistima u dobi 18 do 24 je važan eksterni atribut vina atraktivnost etikete i ambalaže te oznake geografskog porijekla i oznake kvalitete.
Turisti s najvišim dohotkom relativno troše i najveće mjesečne iznose za vino (više od 50 eura), oni s najnižim dohotkom preferiraju slađa vina, češće konzumiraju vino izvan kuće i više su zainteresirani za posjete vinskim podrumima i za tematske ture.

Tablica 4. Korelacije socio demografskih i ekonomskih obilježja turista i preferencija prema atributima vina i ponašanje u konzumaciji vina

	Turisti - Dob			*Turisti - Dohodak*			*Turisti - Spol*		
	χ^2	dF	Cc	χ^2	dF	Cc	χ^2	dF	Cc
Konzumacija vina									
Mjesečna potrošnja	5.1	12	0.462	14.9	12	0.520	0.6	3	0.008
Suho vino	10.5	4	0.310	12.3	4	0.326	0.1	1	0.040
Mjesto konzumacije	15.0	16	0.388	12.2	5	0.476	0.2	1	0.090
Informiranost o vinskim podrumima									
Od prijatelja/poznanika	11.0	4	0.316	11.7	4	0.330	0.4	1	0.065
Iz turističkih agencija	6.1	4	0.186	4.3	4	0.208	0.2	1	0.015
Atributi vina									
Mikro /vinska regija	24.0	20	0.442	30.2	30	0.474	8.37	4	0.271
Terroir / lokalitet	20.9	20	0.418	26.7	30	0.452	10.6	5	0.303
Atraktivnost oznake / imena	35.3	20	0.513	27.0	24	0.454	8.9	4	0.278
Preporuke prijatelja	21.1	14	0.419	22.9	30	0.425	5.4	5	0.221

Izvor: izračun autora

Turisti su u većini varijabli imali slabiju povezanost korelacija u odnosu na lokalno stanovništvo, izuzev u preferenciji prema suhom vinu i to ovisno o dohotku. Značajnije razlike pokazale su se u prepoznavanju vinskih atributa te su dob i spol značajnije kod turista utjecale na stavove o atributima vina u smislu bolje ocjene i veće važnosti „terroir“-a odnosno lokaliteta proizvodnje vina, te mikroregije proizvodnje.

ZAKLJUČAK

Istraživanje razlika u populaciji potrošača vina ukazalo je na izvjesne razlike u populaciji domaćih i inozemnih potrošača. Na uzorku lokalnog stanovništva i turista pokazalo se da postoje razlike u konzumaciji vina i preferencijama prema pojedinim atributima vina.

Intrinzični atributi vina oznaka (geografskog porijekla, oznake izvornosti) te preporuke vina od strane prijatelja i/ili stručnjaka pokazali su se najvažnijim atributima vina. Od ekstrinzičnih atributa važni su vinska regija i terroir. Navedeni atributi pokazali su se značajnijim za skupinu ispitanika lokalnog stanovništva u odnosu na turiste.

U obje skupine ispitanika u konzumaciji vina prevladava preferencija prema suhim vinima te preferencija prema mjestu konzumacije unutar vlastitog doma, međutim lokalno stanovništvo u konzumaciji pokazuje veću varijabilnost prema mjestu konzumacije vina.

Suprotno od očekivanoga lokalno stanovništvo je pokazalo veću motiviranost u odnosu na turiste za sadržaje destinacije kao što su vino, gastronomska ponuda i obilazak povijesnih i prirodnih znamenitosti u Istri.

Vino kao proizvod i dio ponude u turističkoj destinaciji Istra atraktivan je faktor i motiv za dolazak te je prema nalazima istraživanja u odnosu na socio demografski profil za očekivati veću zainteresiranost muškaraca za obilazak vinskih podruma i vinskih cesta te manifestacije vezane uz vino, a od žena za tematske programe koje uključuju vino ali i druge gastronomske sadržaje te razglede povijesnih i prirodnih lokaliteta.

U odnosu na ponašanje u konzumaciji i prihodima ispitanika, potencijal za konzumaciju vina u destinaciji Istra i na vinskim cestama nalazimo u obje skupine ispitanika koji uobičajeno vino konzumiraju u ugostiteljskim objektima. Stoga za skupine nešto niže platežne moći i mlađu populaciju treba organizirati obilazak vinarija s odabirom slađih vina, s naglaskom na ekstrinzične atribute vina. Za skupine višeg obrazovanja i više platežne moći treba usmjeriti ka vinarijama koje proizvode suha vina te dati naglasak na odlike mikroregije i specifičnosti „terroir"-a (naglašeno na pakiranju, buteljke).

Marketinške aktivnosti bi prema navedenim skupinama trebalo usmjeriti kroz promociju cjelokupne destinacije Istra na turističkim i vinskim sajmovima i manifestacijama te kroz društvene mreže, pogotovu za mlađu populaciju i s obzirom na nalaze istraživanja koji ukazuju da uži krug prijatelja i poznanika jako utječe na ponašanje u kupnji i konzumaciji vina.

LITERATURA

www.malvasia-touristra.eu (dosegnuto 28.09.2017.)
www.nn.hr (dosegnuto 28.09.2017.)

Ampuro, O., Villa, N. (2006): Consumers perception of product packing. *Journal of consumers market* 23:100-112.

Bruwer, J., Li, E., Reid, M. (2001): Wine–related lifestyle segmentation of the Australian domestic wine market. The Australian and New Zealand Wine Industry Journal 16(2): 104-108.

Cardebat, J. M., Fiquet, J. M. (2009): Estimation of a hedonic price equation for Alsace Beaujolas and Provence wines. *Applied Economics*, 16(7-9):921-927.

Chrea, C., de Melo, L., Evans, G., Forde, C., Delahunty, C., Cox, D. N. (2011): An investigation using three approaches to understand the influences of extrinsic product cues on consumer behaviour: An example of Australian wines. *Journal of Sensory Studies* 23:13-24.

Crompton, J. L., McCay, S. L. (1997): Motives of visitors attending festival events. Annals of Tourism Research 24(2): 425-439.

Dann, G. (1977): Anomie, ego-enhamcement and tourism. Annals of Tourism Research 4(4), 184-194.

Dann, G. (1981): Tourist motivation: An appraisal, Annals of Tourism Research 8(2), 187-219.

Duarte, F., Madeira, J., Barreira, M. (2010): Wine purchase and consumption in Portugal – an exploratory analysis of young adults' motives/attitudes and purchase attributes, *Ciência Téc. Vitiv.*25(2): 63-73.

Getz D., G. Brown (2006): Critical successs factors for wine tourism regions: a demand analysis. Tourism Management 27:146-158.

Getz, D. (2000): Explore wine tourism: Management, development & destinations. Cognizant Communication Corporation, New York, USA.

Hall, C. M., Macionis, N. (1998): Wine tourism in Australia and New Zealand. (In Butler R, CM Hall and J Jenkins eds.) Tourism and recreation in rural areas. John Wiley & Sons Ltd., Chichester, UK: 197-224.

Hojman, D. E., P. Hunter-Jones (2012): Wine tourism :Chilean wine regions and routes. Journal of Bussiness Reserach 65:13-21.

Ilak Peršurić, A .S., Težak Damijanić, A., Saftić, D. (2016): Winery and wine exhibition visitors' characteristics in the context of wine tourism development. New Medit 4:82-89.

Ilak Peršurić, A. S., A. Červar (2012): Stavovi posjetilaca o izložbi vina „Vinistra" i preferencije prema vinima. Zbornik radova 47. hrvatski & 7. međunarodni znanstveni simpozij agronoma: 175-178.

Outreville, F. J. (2011): Does the bottle size matter? An investigation into differences between posted and market price. *AAWE Working Paper* 86.

Piqueras-Fiszman, B., C. Spence, (2012): The weight of the bottle as a possible extrinsic cue with which to estimate the price (and quality) of the wine? Observed correlations. Food Quality and Preferences 25:41-45.

Sparks, B. (2007): Planning a wine tourism vacation? Factors that help to predict tourist behavioral intentions. Tourism Management 28:1180-1192.

Težak Damijanić, Ilak Peršurić, A. S., Luk, N. (2015): Malvazija Istarska kao sadržaj turističkog proizvoda Istarske županije. Zbornik radova 50. hrvatski simpozij agronoma 179-183.

Yuan, J., Liping, C. A., Morrison, M. A, Linton S. (2005): Segmenting wine festival attendees: A factor – cluster approach. Tourism Review International 8:297-309.

INVESTIGATING RELATIONSHIP BETWEEN FINANCIAL LEVERAGE AND FINANCIAL PERFORMANCE: CASE STUDY – BOSNIA AND HERZEGOVINA BEVERAGE INDUSTRY*

ISPITIVANJE ODNOSA FINANSIJSKE POLUGE I FINANSIJSKIH PERFORMANSI: STUDIJ SLUČAJA – INDUSTRIJA OSVJEŽAVAJUĆIH BEZALKOHOLNIH PIĆA U BOSNI I HERCEGOVINI

Alen Mujčinović[1**], Aleksandra Nikolić[2], Mirza Uzunović[2]

Original scientific paper

Summary

Nowadays, managing a company is becoming more and more complex task. Factors, such as fast-changing environment, highly competitive market put in focus managers' abilities to recognize investment possibilities, establish flexible capital structure and consequently mitigate level of financial risk and contribute to the overall company stability. One way in achieving above mentioned is efficient use of financial leverage. Therefore, the aim of this paper is to investigate whether capital structure/financial leverage positively influences company's financial performance. This question has been discussed for decades, mostly in developed world, while no similar research is done in B&H. The research is done using secondary data from Bisnode BH database for a period of last five years. The sample include 28 companies from beverage industry which is fast-growing and one of the strongest industry in B&H. Regression analysis was used to determine the relationship between the variation in firm value and capital structure. The debt to equity ratio was used as a proxy for capital structure and the following ratios were used for firm value: Net Profit Margin, Return on Assets, Return on Equity, Operative Margin, and Value added per employee. Results of this study will provide valuable inputs for managers of companies as well as potential investors in the sector of beverage industry.

Key words: *financial leverage, firm value, B&H beverage industry*

Rezime

U današnjem vremenu upravljanje kompanijama postaje sve kompleksniji zadatak. Faktori, kao što su rapidne promjene u okruženju, visokokonkurentno tržište stavljaju

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] University of Sarajevo, Faculty of Agriculture and Food Sciences, Student, Bosnia and Herzegovina
[2] University of Sarajevo, Faculty of Agriculture and Food Sciences, Institute of Agricultural and Food Industry Economics, Bosnia and Herzegovina
**corresponding author: mujcinovicalen@hotmail.com

u fokus menadžerske sposobnosti da prepoznaju investicijske mogućnosti, uspostave fleksibilnu strukturu kapitala i kauzalno minimiziraju nivo finansijskog rizika, te utiču na cjelokupnu stabilnost kompanije. Jedan od načina za ispunjenje navedenog jeste efikasno korištenje finansijske poluge. Stoga, cilj ovog rada je ispitati da li struktura kapitala/finansijska poluga pozitivno utiče na performanse poslovanja kompanija. Ova oblast je analizirana decenijama, uglavnom u razvijenim zemljama, dok u BiH ne postoji slično istraživanje. Istraživanje je urađeno korištenjem sekundarnih podataka iz baze podataka Bisnode BH za period zadnjih pet godina. Uzorak čine 28 kompanija iz sektora osvježavajućih bezalkoholnih pića, koji predstavlja najbrže rastuću i jednu od najsnažnijih industrija u BiH. Regresijska analiza je korištena za analizu odnosa između finansijskih performansi i finansijske poluge. Odnos dugovanje imovina je korišten kao indikator strukture kapitala, a sljedeći indikatori su korišteni za analizu finansijskih performansi: neto profitna marža, povrat imovine, povrat kapitala, operativna marža i dodatna vrijednost po zaposlenom. Rezultati ove studije će obezbjediti korisne informacije kompanijama kao i potencijalnim investitorima u sektor osvježavajućih bezalkoholnih pića.

Ključne riječi: *finansijska poluga, finansijske performanse, industrija osvježavajućih bezalkoholnih pića*

INTRODUCTION

"Give me a place to stand,
and I will move the earth."
Archimedes (287-212 BC).

Managing business nowadays is becoming more and more complex task. Companies are bigger, more capital intensive and more competitive on the world market. At the same time, new technologies, price changes (inputs and outputs), changes in supply and demand make management of the companies more cautious and they dedicate special attention to business analysis. They must be able to efficiently use the available resources to achieve predefined goals and at the same time to be ready to respond timely to the different external and internal changes. Successful management of a company as a whole is not easy to learn, it is a complex area that requires cooperation with all stakeholders, chain members, with the goal of finding an "ideal combination" and achieving the best results. In order to achieve above mentioned, operative and strategic decision need to be undertaken, as a one and integral objective. In practice, decisions in most cases cannot be considered separately, because each of them implies the engagement of resources, infrastructural, human, financial, information and other (Đoković, 2013). The role of financial managers in a company is to recognize investment opportunities, analyze and evaluate those opportunities and make decisions on whether and how much to invest. Their aim is to establish a flexible structure of capital that will be able to adapt to alternative financing methods of the enterprise. As all companies are exposed to business risk, the need to find the methods to measure and assess the risk is becoming increasingly apparent (Đoković, 2013).

One of the most important methods is financial leverage which is usually defined as the use of borrowed money to make an investment and return on that investment (Smith, 1990). Aim of this study is to investigate relationship between financial leverage and financial performance of companies operating in B&H beverage industry.

MATERIALS AND METHODS

In attempting to realize the set goals of the business, companies are often forced to borrow capital for which there is a duty to repay. Thus, in order to return a borrowed capital efficiently, companies should use it efficiently in order to generate additional economic benefits. An analysis of the capital structure of an enterprise is carried out using financial indices of indebtedness where the relationship between a foreign and own capital, which is known as financial leverage, plays a key role (Mishra & Modi, 2013). The aim of the leverage is to provide information to the interested parties on how much profitability of a company is sensitive to the changes in key business relationships such as the relationship between variable and fixed costs and the relationship between debt and equity in the balance sheet structure (Welch, 2011). Even though it sounds easy, establishing a proper combination of enterprise financing is a very complex issue and is one of the fundamental issues of financial management. The sample include 28 companies from beverage industry which is fast-growing (increase in production by 9.2 % in 2015), and one of the strongest industries (12% of share in total export of food products) in B&H (MVTEO, 2016). The debt to equity ratio (D/E) was used as a proxy for capital structure and the following ratios were used company's financial performance: Net Profit Margin (NPM), Return on Assets (ROA), Return on Equity (ROE), Operative Margin (OM), and Value added per employee (P). Research model is presented on a figure bellow.

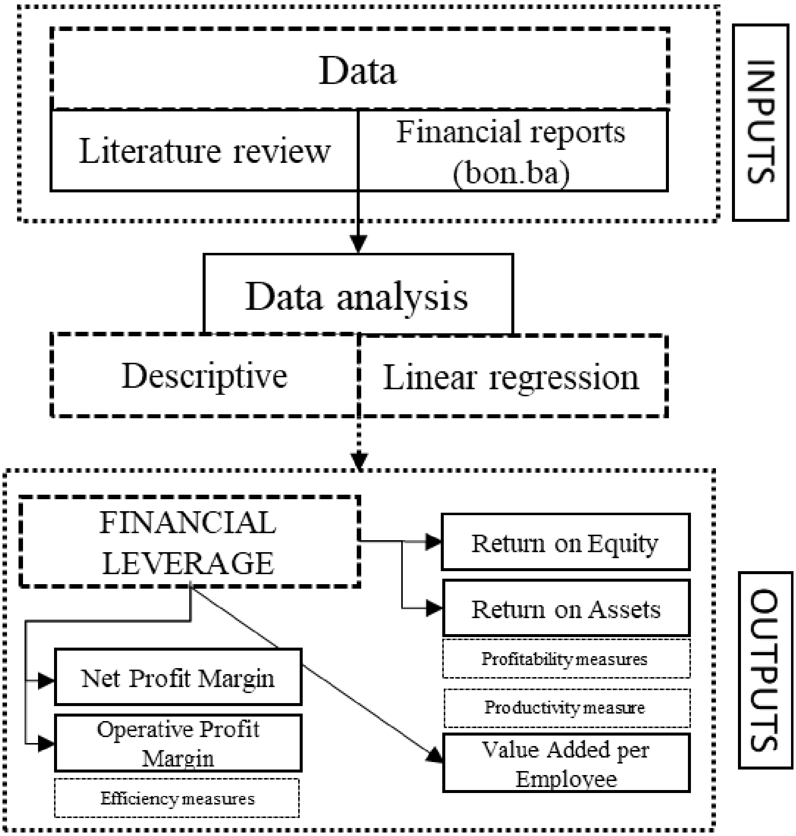


Figure 1 - Research model

Data analysis

This study used Secondary data. The data for the selected stocks were obtained from Bisnode BH database. The leverage of the selected firms was estimated from the annual financial reports covering a period of five years (i.e.2012-2016) of selected 28 companies operating in the beverage sector.

Companies are divided into three categories: small, medium and large in accordance with Accounting and Auditing Law in Federation of Bosnia and Herzegovina (Službene novine FBiH, 83/09) and Accounting and Auditing Law in Republika Srpska (Službeni glasnik RS, 94/15). Capital Structure/financial leverage was the independent variable and profitability, efficiency and productivity ratios were the dependent value. Linear regression analysis was used to determine the relationship between the variation in firm value and capital structure. All statistical data were done using IBM SPSS 22 statistical package.

RESULTS AND DISCUSSION

What is important to remember is the fact that financial leverage serves as measure of risk assessment of a company's business and that they must be used as such in practice. When comparing operating and finance levels between two companies, it is not possible to tell which company is "better" and "worse". The correct comparison is to characterize one of the companies as "more" and the other as "less" risky. There are numerous papers investigating the impact of financial leverage on business performance (Acheampong *et al.*, 2014; Barakat, 2014; Damar *et al.*, 2014; Long & Malitz, 1985; Obradovich & Gill, 2012; Rehman, 2013; Yoon & Jang, 2005). In these researches, the use of financial leverage is recognized as a "two-blade sword" because the company can increase potential gains but also potential losses. The positive relationship between the use of financial leverage and business profitability has been confirmed in the research Modigliani & Miller (1963) as cited in Obradovich & Gill (2012), Jensen (1986), Hann & Smith (2009), Akhtar *et al.* (2012), Ojo (2012), Robb & Robinson (2014). Contrary to the above, some studies have shown a negative link between borrowing and profitability (Akinlo & Asaolu, 2012; Chittenden *et al.*, 1996; Lincoln *et al.*, 1996; Michaelas *et al.*, 2016; Stewart C Myers, 2001; Myers, 1983; Phillips & Sipahioglu, 2004; Sheel, 1994; Wald, 1999). In this study level of financial leverage on companies financial performances were expressed by Return on Assets and Return on Equity as a typical profitability indicators, Net Profit Margin and Operating Margin as typical efficiency indicators and Value Added per Employee as an indicator for productivity. Results from a sample of 28 active companies from beverage industry are presented in table below.

Table 1 – Average level of companies' financial leverage and financial performance indicators (2012-2016)

	FL_2012	FL_2013	FL_2014	FL_2015	FL_2016
total	1,54	1,66	1,75	1,43	1,28
small companies	1,05	1,03	1,02	0,96	0,93
medium companies	3,23	3,65	4,06	2,89	2,52
large companies	0,57	0,78	0,68	0,81	0,53
	NPM_2012	NPM_2013	NPM_2014	NPM_2015	NPM_2016
total	0,03	0,05	0,06	0,07	0,06
small companies	0,03	0,06	0,06	0,08	**0,05**[a*]
medium companies	0,02	0,01	0,04	0,05	**0,06**[b*]
large companies	0,10	0,10	0,12	0,12	0,13
	ROA_2012	ROA_2013	ROA_2014	ROA_2015	ROA_2016
total	0,03	0,04	0,05	0,07	0,04
small companies	0,03	0,04	0,03	0,07	**0,02** [a*]
medium companies	0,02	0,01	0,05	0,04	**0,05** [b*]

large companies	0,11	0,12	0,12	0,10	0,11
	ROE_2012	ROE_2013	ROE_2014	ROE_2015	ROE_2016
total	0,08	0,08	0,10	0,14	0,08
small companies	0,05	0,08	0,05	0,11	0,04
medium companies	0,14	0,08	0,24	0,20	0,15
large companies	0,13	0,14	0,15	0,14	0,14
	OM_2012	OM_2013	OM_2014	OM_2015	OM_2016
total	-.0036	-.0071	0,07	0,11	0,09
small companies	-.0556	-.0572	0,06	0,10	0,07
medium companies	0,06	0,05	0,08	0,10	0,11
large companies	0,16	0,16	0,12	0,16	0,15
	P_2012	P_2013	P_2014	P_2015	P_2016
total	15578,68	49265,39	67241,43	57579,57	53034,32
small companies	13579,83	52114,89	56248,22	61301,33	51443,89
medium companies	9046,57	22136,71	86695,86	34096,86	37172,29
large companies	42813,33	95468,67	87807,00	90042,00	99588,33

Source: Bisnode BH database and author calculation

Legend:
*statistical significant difference found ($p<0.05$)
FL – Financial leverage
NPM – Net Profit Margin
ROA – Return on Assets
ROE – Return on Equity
OM – Operative Margin
P – Value added per employee

As it can be seen from table 1, no statistically significant difference was found between small, medium and large companies and level of financial leverage in observed period. The same result is found for Return on Equity, Operative Margin, Value added per employee. Statistical significant difference was only found in a level of Net Profit Margin indicator for 2016 year between small and medium size companies ($p = 0.044$), and for Return on Assets indicator for 2016 year between small and medium size companies ($p = 0.039$). After this initial testing it was proceeded with testing the relationship between level of financial leverage and firm's financial performance. As it can be seen from following tables, only indicator Return on Equity significantly and positively correlates with level of financial leverage, while all other observed indicators do not have statistically significant correlations.

Table 2 – Results of linear regression model for Net Profit Margin

Model Summary					**ANOVA**		**Coefficients**			
							Unstandardized Coefficients			Standar-dized Coeffi-cients
Model	**R**	R Square	Adjusted R Square	Std. Error of the Estimate	F	**Sig.**	Model	B	Std. Error	**Beta**
NPM_2012	**.140**	.020	-.019	307.948	.503	**.485**	(Constant)	1.865	.720	
							NPM_2012	-8.594	12.113	**-.140**
NPM_2013	**.223**	.050	.013	319.829	1.361	**.254**	(Constant)	2.153	.740	
							NPM_2013	-10.026	8.594	**-.223**
NPM_2014	**.245**	.060	.024	295.289	1.663	**.209**	(Constant)	2.354	.731	
							NPM_2014	-9.789	7.592	**-.245**
NPM_2015	**.161**	.026	-.012	222.831	.690	**.414**	(Constant)	1.741	.567	
							NPM_2015	-4.244	5.111	**-.161**
NPM_2016	**.124**	.015	-.023	217.096	.405	**.530**	(Constant)	1.501	.535	
							NPM_2016	-3.823	6.008	**-.124**

Source: own calculations

From table 2, it was noticed that level of financial leverage negatively influences level of net profit margin (level of Standardized Coefficients – Beta have negative value), but this evidence is not statistically significant (Sig. level above 0.05) for observed period.

Table 3 – Results of linear regression model for Return on Assets

Model Summary					**ANOVA**		**Coefficients**			
							Unstandardized Coefficients			Standar-dized Coeffi-cients
Model	**R**	R Square	Adjusted R Square	Std. Error of the Estimate	F	**Sig.**	Model	B	Std. Error	**Beta**
ROA_2012	**.010**	.000	-.038	3.05455	.002	**.962**	(Constant)	1.526	.685	
							ROA_2012	.546	11.246	**.010**
ROA_2013	**.142**	.020	-.017	3.24754	.537	**.470**	(Constant)	1.927	.717	
							ROA_2013	-6.273	8.558	**-.142**
ROA_2014	**.104**	.011	-.027	3.02935	.284	**.599**	(Constant)	1.962	.701	
							ROA_2014	-4.680	8.787	**-.104**
ROA_2015	**.076**	.006	-.032	2.25105	.153	**.699**	(Constant)	1.521	.490	
							ROA_2015	-1.475	3.773	**-.076**
ROA_2016	**.119**	.014	-.024	2.17220	.375	**.546**	(Constant)	1.468	.511	
							ROA_2016	-4.731	7.729	**-.119**

Source: own calculations

From table 3, it was noticed that level of financial leverage negatively influences level of return on assets (level of Standardized Coefficients – Beta have negative value), but this evidence is not statistically significant (Sig. level above 0.05) for observed period.

Table 4 – Results of linear regression model for Return on Equity

Model Summary					**ANOVA**		**Coefficients**			
							Unstandardized Coefficients			Standar-dized Coeffi-cients
Model	**R**	R Square	Adjusted R Square	Std. Error of the Estimate	F	**Sig.**	Model	B	Std. Error	**Beta**
ROE_2012	**.741**	.549	.532	2.05080	31.685	**.000**	(Constant)	.558	.425	
							ROE_2012	12.001	2.132	**.741**
ROE_2013	**.420**	.177	.145	2.97723	5.575	**.026**	(Constant)	.842	.660	
							ROE_2013	9.742	4.126	**.420**
ROE_2014	**.379**	.144	.111	2.81821	4.370	**.047**	(Constant)	1.177	.598	
							ROE_2014	5.454	2.609	**.379**
ROE_2015	**.381**	.145	.112	2.08763	4.408	**.046**	(Constant)	.913	.464	
							ROE_2015	3.739	1.781	**.381**
ROE_2016	**.294**	.086	.051	2.09114	2.459	**.129**	(Constant)	.811	.497	
							ROE_2016	5.999	3.826	**.294**

Source: own calculations

As already mentioned, only statistical significant correlation was found between level of financial leverage and level of return on equity indicator. A simple linear regression was calculated to predict Return on Equity based on a level of Financial Leverage. A significant regression equation was found for 2012, 2013, 2014, 2015 years. However, only first model in this study were strong enough to examine the relationship between financial leverage and profitability (54.9% of the variance were explained by ROE). For all other models, R^2 values are lower than minimum threshold value of 0.5 and above to have good and acceptable for analysis. It can be seen that positive effects of financial leverage on ROE were reduced from 2012 to 2015 (B value reduced from 12.00 to 3.74) which is a clear sign that companies in B&H tend to decrease efficiency of borrowed resources.

Table 5 – Results of linear regression model for Operative Profit Margin

Model Summary					**ANOVA**		**Coefficients**			
							Unstandardized Coefficients			Standar-dized Coeffi-cients
Model	**R**	R Square	Adjusted R Square	Std. Error of the Estimate	F	**Sig.**	Model	B	Std. Error	**Beta**
OM_2012	**.120**	.014	-.024	3.03270	.378	**.544**	(Constant)	1.548	.573	
							OM_2012	1.122	1.824	**.120**
OM_2013	**.054**	.003	-.035	3.27619	.075	**.786**	(Constant)	1.659	.619	
							OM_2013	.446	1.626	**.054**
OM_2014	**.094**	.009	-.029	3.03221	.234	**.633**	(Constant)	1.880	.636	
							OM_2014	-1.867	3.858	**-.094**
OM_2015	**.047**	.002	-.036	2.25519	.057	**.813**	(Constant)	1.521	.584	
							OM_2015	-.893	3.742	**-.047**
OM_2016	**.124**	.015	-.022	2.17078	.409	**.528**	(Constant)	1.513	.546	
							OM_2016	-2.689	4.204	**-.124**

Source: own calculations

From table 5, it can be noticed that level of financial leverage positively and negatively influences level of operative margin (level of Standardized Coefficients – Beta have positive and negative values), but this evidence is not statistically significant (Sig. level above 0.05) for observed period.

Table 6 – Results of linear regression model for Value Added per Employee

Model Summary					**ANOVA**		**Coefficients**			
							Unstandardized Coefficients			Standar-dized Coeffi-cients
Model	**R**	R Square	Adjusted R Square	Std. Error of the Estimate	F	**Sig.**	Model	B	Std. Error	**Beta**
P_2012	**.011**	.000	-.038	3.05452	.003	**.957**	(Constant)	1.516	.768	
							P_2012	1.755E-006	.000	**.011**
P_2013	**.021**	.000	-.038	3.28022	.011	**.916**	(Constant)	1.699	.742	
							P_2013	-8.768E-007	.000	**-.021**
P_2014	**.021**	.000	-.038	3.04517	.011	**.916**	(Constant)	1.792	.716	
							P_2014	-6.765E-007	.000	**-.021**
P_2015	**.120**	.015	-.023	2.24121	.383	**.541**	(Constant)	1.660	.567	
							P_2015	-4.056E-006	.000	**-.120**
P_2016	**.134**	.018	-.020	2.16819	.472	**.498**	(Constant)	1.545	.560	
							P_2016	-4.944E-006	.000	**-.134**

Source: own calculations

From table 6, it can be noticed that level of financial leverage positively and negatively influence level of value added per employee (level of Standardized

Coefficients – Beta have positive and negative values), but this evidence is not statistically significant (Sig. level above 0.05) for observed period.

CONCLUSION

Owners and management must be aware of financial leverage levels as well as their impact on business in order to timely respond to the both growth and decline in revenues they expect in the future. The business risk is constantly present, there are numerous factors that can reduce the company's potential profits, and as previously emphasized the use of the financial leverage can significantly increase the potential gain but at the same time the potential loss. Therefore, managers, investors and other interested parties are using a wide range of indicators that measure company indebtedness, thereby identifying, assessing, and minimizing potential risks. In this study effect of financial leverage on companies financial performances were analyzed using Net Profit Margin, Operative Margin, Return on Equity, Return on Assets, and Value Added per Employee as key financial performance indicators. These indicators represent efficiency, profitability and productivity indicators and therefore they were suitable for this type of analysis. Results of study identify that only Return on Equity have statistically significant and positive correlation with level of financial leverage. It is also pointed out that positive effects of financial leverage on ROE were reduced from 2012 to 2015 which is a sign that companies in B&H tend to decrease efficiency of borrowed capital. This result could be a warning sign for managers and potential investors in a sector of beverage industry and special attention and efforts should be on an issue how to increase efficiency of borrowed capital.

Main limitation of this study is small sample that may affect results of linear regression model. Also, future studies may extend study period and may also conduct comparative study by taking data from different sectors to check the relationship between financial leverage and financial performances. As already mentioned, research can be done using different proxy for financial risk and compare/asses results and acceptability of methodology.

REFERENCES

Acheampong, P., Agalega, E., & Shibu, A. K. (2014). The Effect of Financial Leverage and Market Size on Stock Returns on the Ghana Stock Exchange: Evidence from Selected Stocks in the Manufacturing Sector. *International Journal of Financial Research*, *5*(1), 125–134. https://doi.org/10.5430/ijfr.v5n1p125

Akhtar, S., Javed, B., Maryam, A., & Sadia, H. (2012). Relationship between Financial Leverage and Financial Performance : Evidence from Fuel & Energy Sector of Pakistan. *International Journal of Economics, Commerce and Management*, *4*(11), 7–18.

Akinlo, O., & Asaolu, T. (2012). Profitability and leverage: Evidence from nigerian firms. *Global Journal of Business Research*, *6*(1), 17–25.

Barakat, A. (2014). The Impact of Financial Structure, Financial Leverage and Profitability on Industrial Companies Shares Value (Applied Study on a Sample of Saudi Industrial Companies). *Research Journal of Finance and Accounting*, *5*(1), 22222847.

Chittenden, F., Hall, G., & Hutchinson, P. (1996). Small firm growth, access to capital markets and financial structure: Review of issues and an empirical investigation. *Small Business Economics*, *8*(1), 59–67.

Damar, H., Farouk, U., & Winarto. (2014). Analysis of the Effect of Financial Leverage and Liquidity Towards Stock Value and Profitability As the Intervening Variable in Trading Companies Listed At Indonesia Stock Exchange 2010-2014. *Jurnal of Business Studies*, 109–116.

Đoković, F. (2013). Business Decision Making On Financing Operating Activites In Hotel Industry. *Socioeconomica – The Scientific Journal for Theory and Practice of Socio-Economic Development*, *2*(3), 67–79.

Službene novine FBiH (2009). Zakon o računovodstvu i reviziji FBiH.

Hann, R. N., & Smith, R. H. (2009). Corporate Diversification and the Cost of Capital, (January).

Jensen. Michael. (1986). Agency Costs of Free Cash Flow, Corporate Finance, and Takeovers. *The American Economic Review*, *76*(2), 323–329.

Lincoln, James, R., Gerlach, Michael, L., & Ahmadjian, Christina, L. (1996). Keiretsu Networks and Corporate Performance in Japan. *American Sociological Review*, *61*(1), 67. https://doi.org/10.2307/2096407

Long, M. S., & Malitz, I. B. (1985). Investment Patterns and Financial Leverage. *Corporate Sapital Structures in the United States*, *I*, 325–352. https://doi.org/10.2307/2328856

Michaelas, N., Chittenden, F., & Poutziouris, P. (2016). Financial Policy and Capital Structure Choice in U. K. SMEs: Empirical Panel Data Evidence from Company. *Small Business Economics*, *12*(2), 113–130.

Mishra, S., & Modi, S. B. (2013). Positive and Negative Corporate Social Responsibility, Financial Leverage, and Idiosyncratic Risk. *Journal of Business Ethics*, *117*(2), 431–448. https://doi.org/10.1007/s10551-012-1526-9

MVTEO. (2016). Godišnje izvješće iz oblasti poljoprivrede, ishrane i ruralnog razvoja za BiH za 2015 godinu. *Ministarstvo Vanjske Trgovine i Ekonomskih odnosa BIH*. Myers, S. C. (1983). The Capital Structure Puzzle. *The Journal of Finance*, *XXXIX*(3).

Myers, S. C. (2001). Capital Structure. *Journal of Economic Perspectives*. https://doi.org/10.1257/jep.15.2.81

Obradovich, J., & Gill, A. (2012). The Impact of Corporate Governance and Financial Leverage on the Value of American Firms. *International Research Journal of Finance and Economics*, *9*, 1–14.

Ojo, A. S. (2012). The Effect of Financial Leverage on Corporate Performance of Some Selected Companies in Nigeria. *Canadian Social Science*, *8*(1), 85–91.

https://doi.org/10.3968/j.css.1923669720120801.700
Phillips, P. A., & Sipahioglu, M. A. (2004). Performance implications of capital structure: evidence from quoted UK organisations with hotel interests. *The Service Industries Journal*, *24*(5), 31–51. https://doi.org/10.1080/0264206042000276829
Rajan, R. G., & Zingales, L. (1995). What do we know about capital structure: some evidence from international data. *J. Finance*, *50*(5), 1421.
Rehman, S. S. F. U. (2013). Relationship between financial leverage and financial performance: Empirical Evidence of listed sugar companies of Pakistan. *Global Journal of Management and Business Reserach Finance*, *13*(8), 33–40. Retrieved from http://journalofbusiness.org/index.php/GJMBR/article/viewFile/1081/992
Robb, A. M., & Robinson, D. T. (2014). The capital structure decisions of new firms. *Review of Financial Studies*, *27*(1), 153–179. https://doi.org/10.1093/rfs/hhs072
Službeni glasnik RS (2015). Zakon o računovodstvu i reviziji Republike Srpske.
Sheel, A. (1994). Determinants of Capital Structure Choice and Empirics on Leverage Behavior: a Comparative Analysis of Hotel and Manufacturing Firms. *Journal of Hospitality & Tourism Research*, *17*(November 2015), 1–16. https://doi.org/10.1177/109634809401700302
Smith, A. (1990). The effects of leveraged buyouts. *Business Economics*, 19-25. Wald, J. K. (1999). How Firm Characteristics Affect Capital Structure: an International Comparison. *The Journal of Financial Research*, *XXII*(2), 161–187. https://doi.org/10.1111/j.1475-6803.1999.tb00721.x
Welch, I. (2011). Two common problems in capital structure research: The financial-debt-to-asset ratio and issuing activity versus leverage changes. *International Review of Finance*, *11*(1), 1–17. https://doi.org/10.1111/j.1468-2443.2010.01125.x
Yoon, E., & Jang, S. (2005). The Effect of Financial Leverage on Profitability and Risk of Restaurant Firms. *The Journal of Hospitality Financial Management*, *13*(1), 35–47. https://doi.org/10.1080/10913211.2005.10653798

RURAL DEVELOPMENT AND FARMERS' SOCIAL EMBEDDEDNESS IN BOSNIA AND HERZEGOVINA*

RURALNI RAZVOJ I SOCIJALNA UKLJUČENOST FARMERA U BOSNI I HERCEGOVINI

Aleksandra Nikolić[1], Mirza Uzunović[1], Alen Mujčinović[2]

Original scientific paper

Summary

Among many ways to determine factors influencing farmers' capabilities to expand, innovate, and improve business activities, stand social embeddedness and social context, which both, drive complex economic, social and political processes, thus defining ability of local actors to activate and use available resources. Both, social embeddedness and context can be viewed as boosting factors of the quality life in rural areas. The aim of this paper is to examine social context and social embeddedness (measured by structural social relations – membership in cooperatives, NGOs etc.) influence on farmers' abilities and intentions to use rural development programs (RDP) in Bosnia and Herzegovina case. For this purpose, the structured questionnaire was developed and distributed in two municipalities (Visoko and Žepče) providing 296 usable surveys. Structural equation modeling along with descriptive statistics was used to analyze farmers' responses. The results show that in Bosnia and Herzegovina low farmers' socio-economic embeddedness restricts access to other capitals, especially natural and cultural, which stands the best chances to foster rural development. Prior participation in rural development programs (RDP), non-farm employment and level of social embeddedness are the main factors influencing farmers' intention to use RDP measures.

Key words: *social embeddedness, social context, rural development policies, Bosnia and Herzegovina*

Rezime

Od mnogo načina da se odrede faktori koji utiču na mogućnosti farmera da šire, inoviraju, unapređuju poslovne aktivnosti nalazi se i socijalna uključenost i socijalni kontekst, od kojih oboje čine kompleksne ekonomske, socijalne i političke procese i definišu mogućnost lokalnih aktera da aktiviraju i koriste resurse. Oboje, socijalna

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina

[1] Faculty of Agriculture and Food Sciences, Institute of Agricultural and Food Industry Economics
[2] Faculty of Agriculture and Food Sciences, Student

uključenost i socijalni kontekst, mogu se posmatrati kao faktori koji podstiču kvalitet života u ruralnim područjima. Cilj rada je istražiti uticaj socijalnog konteksta i socijalne uključenosti (mjerene strukturalnim socijalnim odnosima - članstvo u zadrugama, nevladinim organizacijama itd.) na mogućnosti i namjere farmera da koriste programe podrške ruralnog razvoja (RRP) u slučaju Bosne i Hercegovine. Za ovu svrhu razvijen je i distribuiran strukturirani upitnik u dvije općine (Visoko i Žepče), dajući 296 upotrebljivih upitnika. Strukturalno modeliranje i deskriptivna statistika su korišteni za analizu odgovora farmera. Rezultati pokazuju da niska socio-ekonomska uključenost farmera u Bosni i Hercegovini ograničava pristup ostalim kapitalima, posebno prirodnim i kulturalnim, koji imaju najbolje šanse za unapređenje ruralnog razvoja. Ranije učešće u programima ruralnog razvoja (RRP), zaposlenje van farme i socijalna uključenost su glavni faktori koji utiču na namjeru farmera da koristi RRP mjere.

Ključne riječi: *socijalna uključenost, socijalni kontekst, politike ruralnog razvoja, Bosna i Hercegovina*

INTRODUCTION

Rural areas are often described as an 'mosaic of rural regions with winners, losers and inbetweens' (Terluin, 2003; Woodhouse, 2006). Development of these regions are affected by mutliple factors, while development rate mostly depends on a capacity of community or ability of local actors to activate/use available resources and to organize proper local response to global challenges (Hodge and Midmore, 2008; Nardone *et al.*, 2010; Sabatini, 2009; Righia, 2013). Social context drives all socio-economic interactions among individuals, groups and internal and external networks, and in the same time individuals form groups, networks, build framework for communication and cooperation and knowledge exchange and take economic actions that drives development and creates certain social context. However, there is still lack of information that explains local actor roles, behavior and the way to raise their capacities to access/activate existing resource to become productive part of successful development process, especially in the emerging economies like Bosnia and Herzegovina. Such understanding becomes even more important due to fact that economic logic, monetary and technological incentives alone cannot provide a sufficient incentive for individuals to take action when systems are complex and risk is high (Mariola, 2012; Li *et al.*, 2013). This research main objective is to shed light on social context in which farmers operate and how it affects farmers capability and intention to become an active part of economic recovery of rural areas in Bosnia and Herzegovina. Such insights will contribute to partly fill existing research gap regarding the role of local actors in rural development of emerging countries with weak formal institutional framework. It, also can support policy process that will result in rural or cohesive-like public policies sensitive to characteristics of social context. Such tailor-made policies are likely to be successful to induce and complement development of rural areas in Bosnia and Herzegovina.

THEORETICAL FRAMEWORK AND RESEARCH METHODS

The conceptual and operational approach to identify socio-economic positions of individuals that is shaped by the nature, depth and extent of a local actor tie to a social context is called 'embeddedness'. This research approach s is widely used and in rural literature is "related to being part of the rural environment in both social and economic contexts as well as to the characteristics of the entrepreneurs" (Kjeldsen *et al*., 2012). Social embeddedness shape social interactions that supports flow of information, communication, cooperation and capability to access and activate existing and otherwise unattainable resources (Kim and Kang, 2014; Magnani and Struffi, 2009; Granovetter, 2005; Murray, 2006; Hinrichs, 2000; Moody and White, 2003; Ferru *et al*., 2011). Moreover, level and form of social embeddedness as a reflection of individuals position, power, social relations and capabilities have profound effect on economic performance and shape local capacities to adopt global challenges. Therefore, individual membership in formal and informal networks generates trust and capability to access to otherwise unobtainable resources and, therefore, to act as a promotor of development. It is not easy to capture social embeddedness and its influence on economic output. The overall impact of social context is captured by farmers' intention to invest as it reflects individual capability to recognize business opportunities. In order to capture quality of farmers' social embeddedness (social context in which he operates) and intensity of social interactions the following variables have been considered: *(i) trustworthiness of relations* (measured by farmers willingness to take part in rural development programs (RDP) and personal perception of the RDP corruption level), *(ii) quality and intensity of social relations* (measured by membership in cooperatives or other civic organizations, willingness to cooperate, perception of benefits that can be raised by participation in cooperatives, and self-confidence), and *(iii) level of entrepreneurship* (measured by portion of product sold, intention to stay in the business and participation in RDP). It is also important to mention that role of intermediary organization (such as agribusiness incubator) can have significant influence on a cooperation efficiency, knowledge generation and dissemination (Iturrioz *et al*., 2014). In this study, we test existence of aforementioned effect in two rural areas, first one (Žepče) where agribusiness incubator operates (established through EU development projects), and second one (Visoko) where no such institution exists.

Data collection and analysis

Face-to-face interviews covering 296 farm households in two regions of Bosnia and Herzegovina, Žepče (147 households) and Visoko (149 households) have been organized in the period November 2014 and January 2015. The questioner was structured in three major parts: rural development policies; cooperation and networks;

and farm household management; producing a database with 147 variables measured through a 5-point Likert scale (whereas 1 denoted strong disagreement and 5 strong agreement with the statements). These two municipalities were selected on the basis of similar socioeconomic characteristics of farmers, similar RRP measures and one difference – Žepče has an efficient intermediate organization while Visoko does not. Following the descriptive statistics, an exploratory factor analysis was performed, using Principal Component Analysis and Varimax rotation, with fixed numbers of factors (3). The items with communalities under 0.5 were excluded. Exploratory factor analysis was followed with confirmatory factor analysis. Model was fit through CMIN/DF (1<CMIN/DF>3) and RMSEA (RMSEA < 0.8). Kruskal-Wallis test was used to test differences among organizational membership across regions. The data were processed using the PLS SEM (Partial Least Squares Structural Equation Modeling) and MGA (MultiGroup Analysis).

RESEARCH RESULTS AND DISCUSSION

As it was expected farmers from first region in which intermediary organization operates are significantly more willing to take part in social life (over 70% of them are members of any organization). The majority of those farmers are members of cooperatives. So, the work-related expectations (perceived benefits) are behind farmers' motivation to become active part of the society. Such research results suggest that intermediary organization was able to support cooperation by ensuring economic benefits to the active participants (better access to market, value added and better export prices).

Table 1. Application to Rural Development Programs

		Žepče			Visoko			Total		
		None	Coop.	Other	None	Coop.	Other	None	Coop.	Other
Number of respondents		44	82	21	132	4	13	176	86	34
Age		49.52	49.26	46.67	50.82	48.5	50.85	50.49	49.22	48.26
Education (%)	Primary school (4 yrs.)	2.27	2.44	0.00	3.79	0.00	0.00	3.41	2.33	0.00
	Primary school (8 yrs.)	6.82	15.85	9.52	9.09	0.00	0.00	8.52	15.12	5.88
	High school (3-4 yrs.)	86.36	75.61	66.67	81.82	75.00	100.00	82.95	75.58	79.41
	College (2 yrs.)	4.55	2.44	14.29	1.52	0.00	0.00	2.27	2.33	8.82
	University (4 yrs.)	0.00	3.66	9.52	3.79	25.00	0.00	2.84	4.65	5.88
Applied for RDP support in the last 3 years (%)		40.91	47.56	23.81	43.94	75.00	61.54	43.18	48.84	38.24
How do you assess your intention to use the RDP for your household in the next 3-5 years? (1 no intention, 5 high likelihood)		3.23	3.27	3.48	3.14	3.25	3.54	3.16	3.27	3.5
I intend to apply for the RDP in one of the next calls. (1 no intention, 5 high likelihood)		3.75	3.68	3.76	3.39	3.25	3.77	3.48	3.66	3.76
Do you plan to invest on your farm in the next 3-5 years? (1 no intention, 5 high likelihood)		3.09	3.57	3.48	3.37	3.25	3.38	3.3	3.56	3.44

Legend:
None-not member of any type of organization; Coop.-member of cooperation type of organization; Other-member of any other type of organization

Group of farmers engaged in various voluntary organizations and NGO reported significantly higher perception of benefits that any kind of organization can raise (usefulness, contribution to development, accessibility of credits etc.). This is in line with previous research results which point out that voluntary organizations foster the diffusion of civicness and cooperative values (Sabatini, 2009). Also, non-members have a very high expectation regarding those benefits raised by organization. It seems that some of those expectations (easy access to credit) are not fully realized by organizations, especially in region without intermediate organization. This is supported by the lower marks given by current members during assessment of membership benefits. However, negative image of organization is generally not so pronounced (average mark is less than 3). In both regions non-members in comparison with members have more pronounced negative opinion about organizations.

Table 2 – Information sources

I get help/information from	Žepče				Visoko				Total			
	Non	Coop	Other	Diff.	Non	Coop	Other	Diff.	Non	Coop	Other	Diff.
Family members	3.20	2.82	2.00	0.002	2.63	3.00	2.69	0.855	2.77	2.83	2.26	0.083
Other people from the village	2.82	2.73	2.19	0.116	2.76	3.75	3.31	0.125	2.77	2.78	2.62	0.737
Members from NGO	1.16	2.23	2.86	0.000	1.45	3.00	1.92	0.002	1.38	2.27	2.50	0.000
Members from cooperative	1.32	2.88	3.52	0.000	1.50	3.25	1.69	0.004	1.45	2.90	2.82	0.000
Members from professional organizations	1.09	1.66	2.24	0.001	1.34	2.75	1.15	0.001	1.28	1.71	1.82	0.006
National extension agents	1.07	1.30	1.14	0.264	1.30	2.00	1.77	0.030	1.24	1.34	1.38	0.832
Local ministry units staff	1.27	1.49	1.52	0.278	1.53	2.25	1.38	0.139	1.47	1.52	1.47	0.848
Local government & municipality staff	1.48	1.94	2.05	0.043	2.44	3.50	2.46	0.197	2.20	2.01	2.21	0.271
Private consultants	1.34	1.43	1.52	0.508	1.48	1.75	2.15	0.062	1.45	1.44	1.76	0.098
Media (TV, radio, internet, newspapers)	2.23	2.22	1.71	0.364	2.90	3.50	2.62	0.548	2.73	2.28	2.06	0.004
International development projects	1.16	1.30	1.86	0.002	1.41	2.25	1.54	0.084	1.35	1.35	1.74	0.047

In both regions farmers still relay on close personal networks consisted on family and close friends - capability of current web formal and non-formal networks to enhance information flow and increase communication and cooperation is still under developed. The external sources of information such as media, private consultants and government and municipality staff are not considered as a trustful source of information. This suggest poor relational embeddedness shaped by low trust in weak local formal institutions (state administration, extension services and municipality staff).

Participants who are members of cooperation have significantly higher perception of benefits that any kind of organization can raise (usefulness, contribution to development, accessibility of credits etc.). Voluntary organizations foster the diffusion of civicness and cooperative values (Sabatini, 2009). Participants, non-members have more pronounced negative opinion about organizations. Some of expectations (easy access to credit) are not fully realized by organizations, especially in region without intermediate organization.

Table 3 – Attitudes towards organizations

	Žepče				Visoko				Total			
	Non	Coop.	Other	Diff.	Non	Coop.	Other	Diff.	Non	Coop.	Other	Diff.
In general, membership in an organization is useful	3.75	4.44	4.62	0.000	3.80	3.00	4.38	0.020	3.78	4.37	4.53	0.000
My family approves membership in an organization	4.07	4.41	4.67	0.000	3.89	3.75	4.31	0.137	3.94	4.38	4.53	0.000
People I respect approve membership in an organization	3.91	4.16	4.52	0.003	3.70	3.50	4.15	0.108	3.76	4.13	4.38	0.000
Organizations contribute to the development of the village	3.73	4.15	4.57	0.000	3.66	3.75	4.00	0.243	3.68	4.13	4.35	0.000
Organizations provide assistance when preparing for RDP application	3.59	3.76	4.10	0.059	3.34	3.25	3.77	0.074	3.40	3.73	3.97	0.000
Members of organizations get bank credit more easily	3.55	3.46	3.33	0.852	3.33	3.25	3.46	0.795	3.38	3.45	3.38	0.706
People in organizations only think of themselves and their interest	3.16	2.67	1.86	0.000	3.08	3.00	2.54	0.053	3.10	2.69	2.12	0.000
Organizations are formed only to use money from funds	2.95	2.49	1.90	0.000	3.11	2.50	2.54	0.003	3.07	2.49	2.15	0.000
I believe that members in organizations respect joint agreements	3.16	3.48	4.29	0.000	3.19	3.00	3.46	0.294	3.18	3.45	3.97	0.000
Being a member in an organization, does not stop me to freely decide for my farm	4.23	4.32	4.43	0.651	3.70	3.75	3.92	0.796	3.83	4.29	4.24	0.000
The process of joint decision-making works well	3.52	3.89	4.24	0.003	3.25	3.25	3.62	0.146	3.32	3.86	4.00	0.000

Second part of the study aimed to identify relationship between intention to invest on farm and three variables of social embeddedness named: trustworthiness of relations, quality and intensity of social relations and level of entrepreneurship. The structural equation model has been developed for total sample, member and non-member groups and main results are presented in following table.

Table 4. Results of SEM analysis for total sample, member and non-member groups

Variables	Non-members		Members		Total		Factors	Non-members		Members		Total	
	R	p	R	p	R	p		R	p	R	p	R	p
Trustworthiness of relations	0.160	0.510	0.392	0.001	0.225	0.027	I am able to get credit	0.170	0.730	0.565	0.001	0.569	0.009
							I am confident to get RDP support	0.504	0.298	0.785	0.000	0.785	0.000
							I intend to apply for the RDP in one of the next calls	0.892	0.058	0.686	0.000	0.745	0.000
							RDP approval system is not corrupted	0.470	0.363	0.727	0.000	0.691	0.001
Quality and intensity of social relations	-0.071	0.725	0.257	0.289	-0.079	0.579	I cooperate with other producers	-0.348	0.516	0.849	0.210	-0.772	0.266
							Self-evaluation of the farmers to the others	0.084	0.846	0.832	0.228	-0.785	0.254
							Primary occupation	0.905	0.108	0.464	0.193	-0.015	0.967
Level of entrepreneurship	0.220	0.293	0.232	0.191	0.241	0.131	Intention to farm in future	0.980	0.135	0.979	0.051	0.978	0.039
							Portion of products sold on market	0.387	0.460	0.371	0.384	0.393	0.304
							I applied for RDP	-0.522	0.360	0.042	0.919	-0.296	0.402

Legend:

R – correlation coefficient (- indicate negative correlation)

P – significance level ($p<0.05$ indicate statistically significant influence)

CONCLUSIONS

B&H farmers socio-economic embeddedness is low; their socioeconomic networks are underdeveloped, based on short bonding ties. The outsiders (external stakeholders e.g. consultants, media etc.) are not seen as a trusted source of information. Accordingly, relational ties, that are a basis for knowledge and technology transfer, are not developed. Weak formal institutions, especially local and municipality administration (UNDP, 2012) are not able to generate trust, give framework for economic activities, set rules, provide credibility for business undertaking and reduce risk. Therefore, their capability to foster information flow, communication and cooperation is questionable. Farmers' networks do not support social interaction and knowledge, technology transfer among local and external networks, from which deficient resources/knowledge can be obtained and retained. Because of the poor quality of B&H farmers social relations perception of risk, according to (Traikova *et al*., 2007) farmers can start to doubt their personal capability to handle task of starting/expending business, which includes complicated administrative procedure, "risky search" for financial funds, suppliers and market. Intermediate organizations, such agribusiness incubators could be efficient solution to foster farmers' capability to take risk, spot and seize business opportunities and support development of rural community capability to face local, regional and global challenges. Another efficient solution to foster farmers' capabilities would be an efficient use of social networks.
The research limitation is related to the fact that research was not able to take into consideration heterogeneity of members and the degree of their involvement in types of associations that were considered by research. Also, the research has been more focused on structure of networks and less on quality of relations which have more pronounced impact on individuals' capacity and behavior (Li *et al*., 2013). Also, all differences of social context of two investigated communities were ignored.

REFERENCES

Alessandra Righia (2013): Measuring social capital: Official statistics initiatives in Italy, Procedia - Social and Behavioral Sciences 72, pp 4-22.
Andrew Woodhouse (2006): Social capital and economic development in regional Australia: A case study, Journal of Rural Studies 22, pp 83–94.
Byung-Yeon Kim, Youngho Kang (2014): Social capital and entrepreneurial activity: A pseudo-panel approach, Journal of Economic Behavior & Organization 97, pp 47-60.
C. Clare Hinrichs (2000): Embeddedness and local food systems: notes on two types of direct agricultural market, Journal of Rural Studies 16, pp 295-303.
Chris Kjeldsen, Gunnar Lind, Haase Svensen (2011): Networking private entrepreneurs in rural areas – social capital or waste of time?, Journal of Depopulation and Rural Development Studies, no 11, pp 7-28.

Cristina Iturrioz, Cristina Aragón, Lorea Narvaiza (2014): How to foster shared innovation within SMEs' networks: Social capital and the role of intermediaries, European Management Journal.

Diana Traikova, Judith Möllers, Jana Fritzsch and Gertrud Buchenrieder (2007): Some Conceptional Thoughts on the Impact of Social Networks on Non-farm Rural Employment, 104th Seminar, September 5-8, 2007, Budapest, Hungary from European Association of Agricultural Economists.

Fabio Sabatini (2009): Social capital as social networks: A new framework for measurement and an empirical analysis of its determinants and consequences, The Journal of Socio-Economics 38, pp 429–442.

Gianluca Nardone, Roberta Sisto, Antonio Lopolito (2010): Social Capital in the LEADER Initiative: a methodological approach, Journal of Rural Studies 26, pp 63–72.

Granovetter, M. (2005): The Impact of Social Structures on Economic Outcomes. Journal of Economic Perspectives 19(1), 33-50.

Ian Hodge, Peter Midmore (2008): Models of Rural development Approaches to Analysis Evaluation and Decision-Making, Economie Rurale 307, pp 22-38.

Ida J. Terluin (2003): Differences in economic development in rural regions of advanced countries: an overview and critical analysis of theories, Journal of Rural Studies 19, pp 327–344.

James Moody, Douglas R. White (2003): Structural Cohesion and Embeddedness: A Hierarchical Concept of Social Groups, American Sociological Review 68(1), pp 103-127.

Matt J. Mariola (2012): Farmers, trust, and the market solution to water pollution: The role of social embeddedness in water quality trading, Journal of Rural Studies 28, pp 577-589.

Marie Ferru, Michel Grossetti, Marie-Pierre Bès (2011): How to identify social ties in innovation partnerships? The case of French science-industry collaborations, Paper provided by European Regional Science Association in its series ERSA conference papers with number ersa11p971.

Murray, C. (2006): Social Capital and Cooperation in Central and Eastern Europe, Towards an Analytical Framework, Poster paper presented at the International Association of Agricultural Economists Conference, Gold Coast, Australia.

Natalia Magnani, Lauro Struffi (2009): Translation sociology and social capital in rural development initiatives. A case study from the Italian Alps, Journal of Rural Studies 25, pp 231-238.

UNDP (2012): The Ties that Bind, Social Capital in Bosnia and Herzegovina, can be downloaded at http://hdr.undp.org/en/content/ties-bind.

Yongqiang Li, Xuehua Wang, Lily Huang, Xuan Bai (2013): How does entrepreneurs' social capital hinder newbusiness development? A relational embeddedness perspective, Journal of Business Research 66, pp 2418-2424.

COMPETITIVENESS OF DRINKING WATER AND BEER SECTORS OF BOSNIA AND HERZEGOVINA *

KONKURENTNOST SEKTORA VODE ZA PIĆE I SEKTORA PIVA BOSNE I HERCEGOVINE

Adem Prnjavorac[1], Aleksandra Nikolić[2], Alen Mujčinović[1], Mirza Uzunović[2]

Original scientific paper

Summary

The effects of Bosnia and Herzegovina's liberal trade policies have been unknown and unexamined in specific sectors, like beer and drinking water sectors. Has Bosnia and Herzegovina produced positive effects of main aim of trade liberalization – creating more competitive and more productive sector, question is that remains unanswered? Thus, the aim of this paper is to analyze the competitiveness of beer and drinking water sectors on international markets of Croatia, Turkey, CEFTA members (Serbia, Albania, Macedonia, and Montenegro) as well as EU member countries. The competitiveness level was determined using RCA (revealed comparative advantage) index, categorizing two-way trade flows and Grubel-Lloyd IIT (GLIIT) index during the period from 2008 to 2015. International trade data was obtained using UNCATD database. RCA index shows competitive advantages on markets of Montenegro, Serbia and EU member states for drinking water sector, and on markets of Croatia, Serbia, Montenegro and EU member states for beer sector. Trade flow categorization and GLIIT index show competitiveness based on price competition and strong inter-industry trade relations. Overall results indicate that the future policy should encourage industry to improve the quality characteristics of products.

Key words: *Bosnia and Herzegovina, beer sector, drinking water sector, competitiveness*

Rezime

Efekti liberalizacije tržišta u Bosni i Hercegovini su nepoznati i neistraženi za pojedine sektore, a neki od njih su sektor vode za piće i sektor piva. Jedno od pitanja jeste: da li je glavni efekat liberalizacije tržišta imao pozitivan uticaj na razvoj sektora – kreiranje konkurentnijeg i produktivnijeg sektora? Stoga, cilj ovog rada je analizirati konkurentnost sektora vode za piće i sektora piva na internacionalnom tržištu

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina

[1] Faculty of Agriculture and Food Sciences, Student
[2] Faculty of Agriculture and Food Sciences, Institute of Agricultural and Food Industry Economics

Hrvatske, Turske, članica CEFTA-e (Srbija, Albanija, Makedonija i Crna Gora), kao i zemalja članica EU. Nivo konkurentnosti je analiziran korištenjem RCA (otkrivena komparativna prednost) indeksa, kategorisanjem dvosmjernih trgovinskih tokova i Gruber-Loydovim IIT (GLIIT) indeksom za period od 2008. do 2015. godine. Podaci o internacionalnoj trgovini su preuzeti sa UNCATD baze podataka. Vrijednost RCA indeksa pokazuje konkurentsku prednost sa tržištima Crne Gore, Srbije i zemljama članicama EU za sektor vode za piće i na tržištu Hrvatske, Srbije, Crne Gore i zemalja članica EU za sektor piva. Kategorisanje dvosmjernih trgovinskih tokova i GLIIT indeks ukazuju da je konkurentnost bazirana na cijeni i snažnim inter-industrijskim trgovinskim odnosima. Ukupni rezultati ukazuju da buduća politika treba podsticati unapređenje kvalitativnih karakteristika proizvoda analizirane industrije.

Ključne riječi: *Bosna i Hercegovina, sektor piva, sektor vode za piće, konkurentnost*

INTRODUCTION

The competitiveness of the company, the economic sector and the overall economy is attractive field of study that seeks attention of both the researchers and the wider public. It is linked to economic success and improving the quality of life of each individual, or the welfare of an economy as a whole. Competitiveness is important for countries such as Bosnia and Herzegovina (B&H) that implement a liberal trade policy because the economy is successful if it can respond to the requirements of the free market. The issue of competitiveness is extremely important for B&H given the very low standard of living and high foreign trade deficit. In order to create efficient public and private policies that can dealt with this issue it is necessary to analyze current level of competitiveness but also to predict future changes. Sensitive economic sectors, those who, due to their structural characteristics, technical and technological equipment, and the nature of their work are unable to adapt to the specific conditions in the short term, can be brought in a very difficult position, start to reduce production and economic activities of the country, and therefore increase unemployment, which can slow down the development and endanger the quality of life in country. Agricultural and food industry sector is one of the sensitive sectors and it is expected that these sectors face problems to handle the changes that came as a result of integration and liberalization of foreign trade. Therefore, the aim of this paper is to determine competitive advantages for drinking water and beer sectors on international markets, for B&H‘s main international trade partners.

MATERIALS AND METHODS

Competitiveness is one of the most powerful concepts of modern economy and is a field of study that seek for attention of experts as well as academic society for decades (Nikolić, 2008). In order to measure achieved competitive advantage we use an integral Competitiveness Index, developed for the purpose of this research, and it represent continuation of the research carried out by Uzunović (2010). The Integral Competitiveness Index is calculated on the basis of the calculated Revealed Comparative Advantage Index (RCA); the intra-industrial trade index (Grubel-Lloyd Intra-Industry Trade - GLIIT) and the categorization of trade flows, one-way and two-way trade flows. In order to calculate the integral Competitiveness Index, it is necessary to calculate the above-mentioned indices first, therefore in the following text, we will provide a brief explanation of each of the above indices.

Revealed Comparative Advantage Index - RCA

RCA index identifies strong and less powerful export sectors of the country, and it is an ex-post method to measure competitive advantage. It measures share of one sector in a particular market, hence it belongs to a group of indicators that measure competitiveness based on market share. The RCA index is a static measure of competitive abilities, i.e. giving a picture of competitiveness in one moment. It is developed by Balassa (1965), and it is widely used measure of competitiveness (Abdul Malek *et al.*, 2015; Bahta & Jooste, 2005; Banterle *et al.*, 2014; Bojnec & Ferto, 2006, 2007a, 2007b, 2009a, 2009b; Stefan Bojnec & Imre Ferto, 2014; S. Bojnec & I. Ferto, 2014; Bojnec & Ferto, 2015a, 2015b; Brander & Spencer, 2015; Herciu, 2013; Kocourek, 2015; Marius-Razvan & Camelia, 2015; Nikolić *et al.*, 2011; Utkulu & Seymen, 2004; Yoshida, 2013). RCA index is calculated on a following way:

$$RCA = \frac{\frac{x_{ij}}{x_{it}}}{\frac{x_{nj}}{x_{nt}}}$$

Legend:

x – Export value,
i – Country (B&H),
j – SITC or industry,
t – Goods or industry,
n – Export market.

Thus, the RCA shows the exports ratio value (goods, industry, and sector) in a total value of the country's exports, as well as imports ratio value of products or industry (sectors) in total imports of the country. RCA index values range from 0 (no competitive advantage) to infinite, while RCA index values greater than 1 showing a competitive advantage. The RCA index has some limitations, first as it is giving only a current level of competitiveness, and the second limitation as it is not explaining the reason for the achieved level of competitive advantage. Using data from long period of time can partly overcome first limitation. In order to overcome the second limitation, additional competitiveness indices should be used.

Grubel-Lloyd IIT – GLIIT

Grubel-Lloyd IIT is considered as an indicator of economic integration between countries with similar economies. GLIIT shows whether trade flows of international trade are intra or inter-industrial type, and it is calculating on a following way (Banterle and Carraresi (2007); Uzunović (2010); Nikolić *et al.* (2011):

$$\boldsymbol{GLIIT = 1 - \frac{\sum |X_{ij} - M_{ij}|}{\sum |X_{ij} + M_{ij}|} * 100}$$

Legend:
X – Export value,
M – Import value,
i – Country (B&H),
j – Goods, industry or SITC.

The value of the GLIIT index ranges from 0% (inter-industrial trade) to 100% (intra-industrial trade). Due to an easier interpretation of the GLIIT index Banterle and Carraresi (2007) divided GLIIT index into four categories:

- 0 < GLIIT ≤ 25% - strong inter-industrial trade tendencies,
- 25 < GLIIT ≤ 50% - weak inter-industrial trade tendencies,
- 50 < GLIIT ≤ 75% - weak intra-industrial trade tendencies,
- 75 < GLIIT ≤ 100% - strong intra-industrial trade tendencies.

Thus, the GLIIT index shows the level of sector or industry specialization on the international market and is therefore often used as a supplement to the RCA index. Like the RCA index there are several shortcomings. The limitation is same as for the RCA index, showing a picture of one moment. The GLIIT index only slightly reveals the reasons for the achieved competitive advantages (it gives an answer whether it is an intra or inter-industrial trade).

Categorization of trade flows – KAT

Bojnec and Ferto (2006), Bojnec and Ferto (2007a), and Bojnec and Ferto (2007b) develop and use the method of categorizing trade flows in order to separate price and non-price competitiveness at the international level. The method of categorizing trade flows identifies one-way and two-way trade flows. One-way trade flows only imply import or export trade flows, and two-way trade flows include four categories of trade flows: K1 - successful price competitiveness; K2 - unsuccessful price competitiveness; K3 - successful non-price competitiveness; and K4 - unsuccessful non-price competitiveness. Categories are made on trade balance and unit prices of trade goods. Conditions for separate one-way categories are as follows:

$$Only\ import\ category – TB_{(i,j)} > 0\ \left(ili\ V_{(i,j)}^{x} > 0, \quad V_{(i,j)}^{m} = 0\right)\ i\ UV_{(i,j)}^{m} = 0,$$

$$Only\ export\ category – TB_{(i,j)} < 0\ \left(ili\ V_{(i,j)}^{x} = 0, \quad V_{(i,j)}^{m} > 0\right)\ i\ UV_{(i,j)}^{x} = 0.$$

Conditions for separate two-way categories are as follows:

$$K1 - TB_{(i,j)} > 0 \left(ili\ V_{(i,j)}^{x} > V_{(i,j)}^{m}\right) and\ UVD_{(i,j)} < 0 \left(ili\ UV_{(i,j)}^{x} < UV_{(i,j)}^{m}\right),$$
$$K2 - TB_{(i,j)} < 0 \left(ili\ V_{(i,j)}^{x} < V_{(i,j)}^{m}\right) and\ UVD_{(i,j)} > 0 \left(ili\ UV_{(i,j)}^{x} > UV_{(i,j)}^{m}\right),$$
$$K3 - TB_{(i,j)} > 0 \left(ili\ V_{(i,j)}^{x} > V_{(i,j)}^{m}\right) and\ UVD_{(i,j)} > 0 \left(ili\ UV_{(i,j)}^{x} > UV_{(i,j)}^{m}\right),$$
$$K4 - TB_{(i,j)} < 0 \left(ili\ V_{(i,j)}^{x} < V_{(i,j)}^{m}\right) and\ UVD_{(i,j)} < 0 \left(ili\ UV_{(i,j)}^{x} < UV_{(i,j)}^{m}\right),$$

where trade balance ($TB_{(i,j)}$) is calculated as $TB_{(i,j)} = V_{(i,j)}^{x} - V_{(i,j)}^{m}$ where $V_{(i,j)}^{x}$ is export value for good i to trade partner j and $V_{(i,j)}^{m}$ is import value for good i from partner j. Unit price difference is calculated as $UVD_{(i,j)} = UV_{(i,j)}^{x} - UV_{(i,j)}^{m}$ where $UV_{(i,j)}^{x}$ is unit export price calculated as $UV_{(i,j)}^{x} = \frac{V_{(i,j)}^{x}}{Q_{(i,j)}^{x}}$ and $UV_{(i,j)}^{m}$ unit import price calculated as $UV_{(i,j)}^{m} = \frac{V_{(i,j)}^{m}}{Q_{(i,j)}^{m}}$. $Q_{(i,j)}^{x}$ and $Q_{(i,j)}^{m}$ represent export and import quantities, respectively. Trade balance determines successful or unsuccessful competitiveness, while unit price values determine competitiveness based on product price or product quality. The main limitation of the categorization of trade flows is reflected, as with the RCA and GLIIT index, in giving it a picture of only one moment. To overcome this deficiency, it is recommended to use a time series of international trade data.

Integral Competitiveness Index

This index attempts to overcome all the disadvantages and constraints that RCA, GLIIT and the category of trade flows carry out, and in the same time to use of all the good sides of each individual index. In other words, integral Competitiveness Index should show the sector, industry or goods that have a competitive advantage and determine whether the competitive advantage is achieved on the basis of price or non-price characteristics of the trade exchange. In addition, using the GLIIT index shows whether it is an intra or inter-industrial trade exchange. The Competitiveness Index, therefore, represents a more rigorous measure of competitiveness than the use of combinations or individual indices explained above. In other words, in order to identify the tariff, good or sector as competitive, it is necessary to show competitive advantage in the same sector, tariff or a good RCA index, the GLIIT index weak or strong intra-industrial flows, and that the tariff, good or sector belongs to the favorable trade categories (one-way or two-way). Way of calculating the Competitiveness Index includes calculating RCA, GLIIT and the category of trade flows, and then computing the Competitiveness Index. After calculating the RCA index values, the GLIIT index and the categorized trade flows, the same values are presented graphically, as in figure 1. For the graphical representation, it was necessary to categorize the calculated values of the indexes in the graphical representation. The categorization of the calculated index values is done according to Table 1. In the graphical representation of the RCA index category, the GLIIT index and the category of trade flows, two values of the same are represented: (i) Actual calculated values - represented by the internal troughs closing the values of these indices; and (ii) the optimal or the most favorable values these indices may take-ups - represented by external triangles that close these index values.

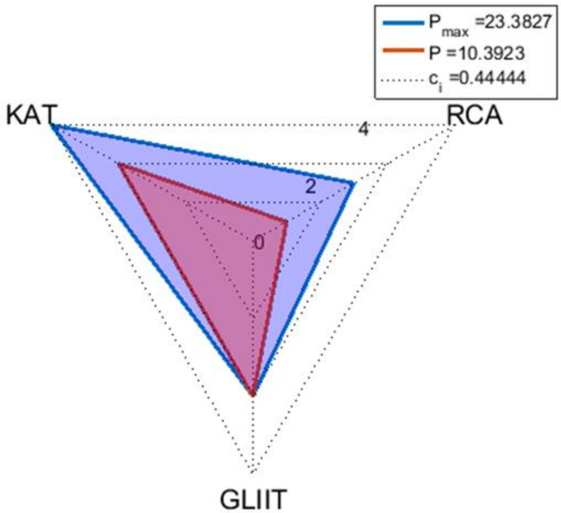


Figure 1 – Competitiveness Index calculation

A graphical representation of the actual and optimal values of the calculated indices is the basis for calculating the Competitiveness Index. The Competitiveness Index is actually the ratio of surfaces that close the two triangles in the graphical representation of RCA, GLIIT index and categories of trade flows. Thus, the Competitiveness Index is obtained as the ratio of the surface of the triangle of the calculated index values (RCA, GLIIT, and the categories of trade flows) categorized according to Table 1 and the triangle surfaces closing the optimal index values, also categorized according to Table 1, that is, index values that are in the most distant place from the source of the system.

The Competitiveness Index values range from 0 (the calculated surface of triangle is zero), to 1 (the calculated surface of triangle of the calculated index is equal to the surface of triangle of the optimal index values). In order to facilitate the interpretation of the value of the Competitiveness Index, it was necessary to introduce the categories of calculated Competitiveness Index, and the suggestion of the categories is as follows:

Table 1 – Competitiveness Index Categorization

Competitiveness Index Values	Name of the category	Explanation
0.00 – 0.25	I category	the lack of competitive advantage in the observed sector, only the import category or the expressed unsuccessful competitive advantages based on product prices
0.25 – 0.50	II category	lack of competitive advantages with the existence of two-way trade flows based on the inter-industrial exchange of the sector
0.50 – 0.75	III category	the existence of competitive advantages of two-way trade flows based on the price characteristics of products, with the existence of both intra and inter-industrial trade flows
0.75 – 1.00	IV category	the existence of competitive advantages of two-way trade flows based on qualitative product characteristics with intra-industrial trade or only export categories
1.06	Only export category	The tariff shows only export during the period
-1.01	Only import category	The tariff shows only import during the period

On a basis of these categories, it is possible to conclude that this index, good or the industry, is marked with a competitive advantage if the Competitiveness Index is greater than 0.5. One-way trade categories are differentiated with values greater (for only export category – 1.06) and smaller (for only import category – -1.01) than Competitiveness Index for two-way categories. Calculation of the Competitiveness Index was made with the help of the Math Works MATLAB® 2016b software package.

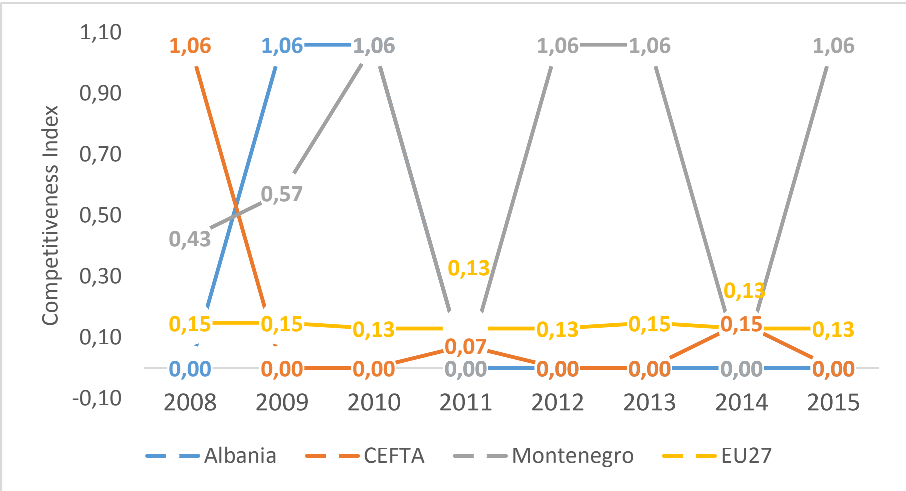
Data sources

The source of data for the calculation of the Competitiveness Index are two databases, database of the Foreign Trade Chamber of Bosnia and Herzegovina and UNCTAD database. From the mentioned databases, the tariffs that comprise the trade exchange of the beer and drinking water sector were selected for the countries: Albania, Montenegro, Macedonia, Croatia, Turkey, Serbia, CEFTA 2006 countries, and the EU27[3]. Countries for the calculation of the Competitiveness Index were selected according to recommendations by Lalić (2010), Uzunović (2010) and Nikolić *et al*. (2011) due to the size, similarity and geographical proximity of the economies of the mentioned countries with the economy of Bosnia and Herzegovina.

[3] Since this is the period from 2010 to 2015, the period when Croatia became a full member of the EU, because of the ease of presentation of results and the importance of Croatia as a trading partner of B&H, Croatia has been omitted from EU Member States even after joining the European Union Union in 2013.

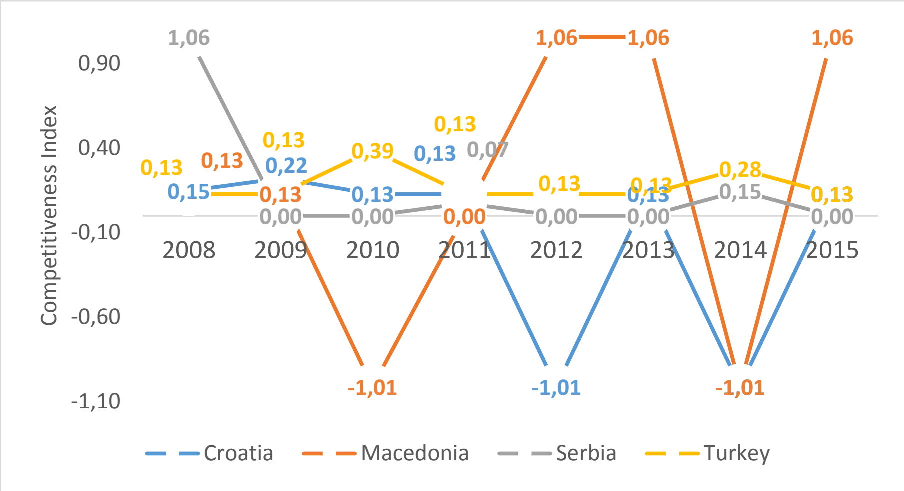
RESULTS AND DISCUSSION

After the calculation, data was presented in the following graphs, which are divided into groups for drinking water and beer sector, as well as for both sectors together. Graph 1 shows Competitiveness Index for Albania, CEFTA, Montenegro and EU27 for drinking water sectors. Drinking water sector is only export sector for markets of Albania and Montenegro, while it shows no competitive advantages on markets of CEFTA and EU27 (Competitiveness Index takes values below 0.5). Stable competitive disadvantage drinking water sector shows on EU27 market, with values around 0.13 through the whole period.



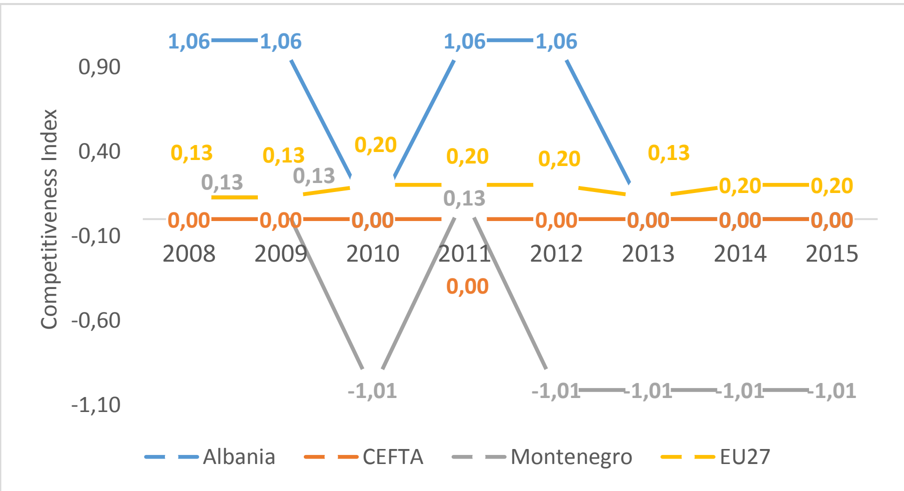
Graph 1 – Competitiveness Index for Water Sector on AU27, CEFTA countries, Albania and Montenegro markets, period 2008-2015
Source: own calculation based on FTCB&H and UNCTAD data

Graph 2 shows Competitiveness Index for drinking water sector on markets of Croatia, Macedonia, Serbia and Turkey. Only on market of Macedonia and Serbia show only export categories, but in an irregular fashion through the period.



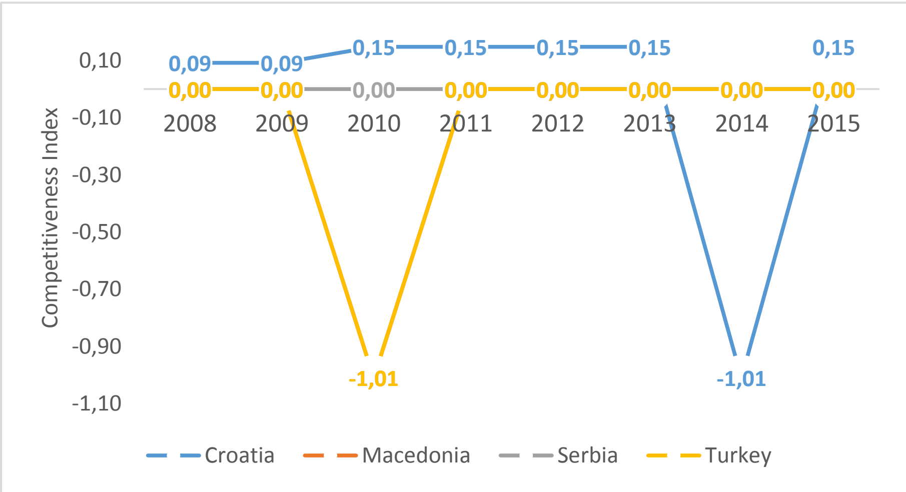
Graph 2 – Competitiveness Index for Water Sector on markets of Croatia, Macedonia, Serbia and Turkey
Source: own calculation based on FTCB&H and UNCTAD data

Competitiveness Index shows the highest values for the market of Turkey, taking values in the second category of Competitiveness Index. For all other markets Competitiveness Index takes values in the first category, explaining two-way trade flows as trade flows with no competitive advantages.



Graph 3 – Competitiveness Index for Beer Sector on AU27, CEFTA countries, Albania and Montenegro markets, period 2008-2015
Source: own calculation based on FTCB&H and UNCTAD data

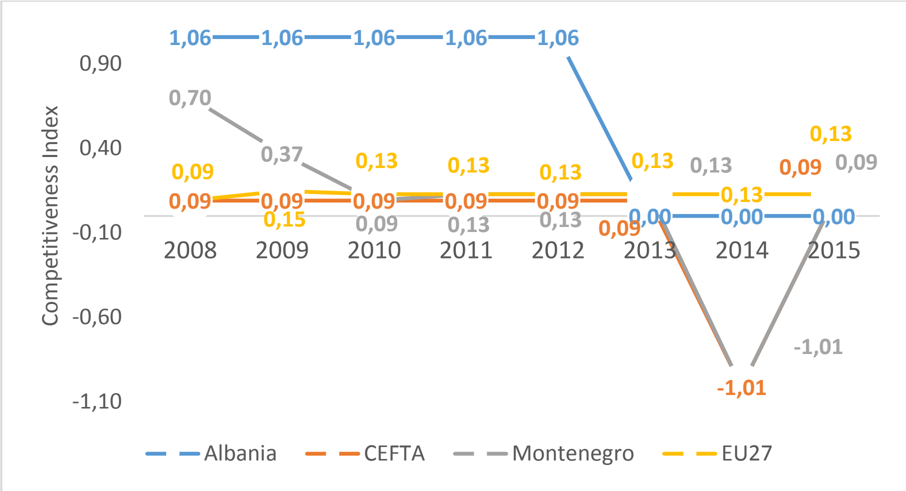
Graph 3 shows Competitiveness Index for beer sector on markets of Albania, Montenegro, CEFTA and EU27. As for the drinking water sector, beer sector shows only export categories on market of Albania. Different from drinking water sector, on market of Montenegro, beer sectors shows only import categories. On other markets, beer sector has no gained competitive advantages.



Graph 4 – Competitiveness Index for Beer Sector on markets of Croatia, Macedonia, Serbia and Turkey

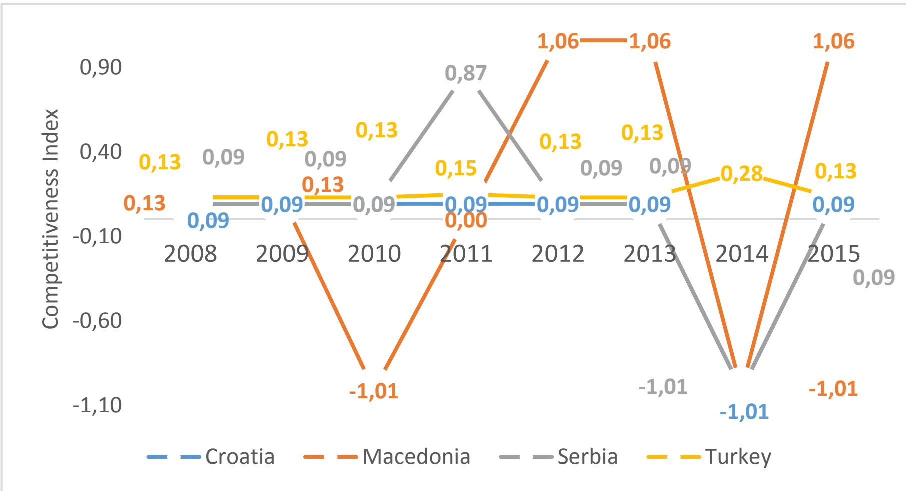
Source: own calculation based on FTCB&H and UNCTAD data

Graph 4 shows Competitiveness Index for Serbia, Croatia, Macedonia and Turkey. For all markets beer sector shows no competitive advantages through the period. The beer sector shows only import categories for markets of Turkey and Croatia in two years of the period. For all other years, Competitive Index values are all the same for each market, excluding market of Croatia, where Competitiveness Index takes the highest values for almost all years.



Graph 5 – Competitiveness Index for drinking water and beer sectors on AU27, CEFTA countries, Albania and Montenegro markets, period 2008-2015
Source: own calculation based on FTCB&H and UNCTAD data

Graph 5 shows Competitiveness Index of both sectors together on markets on Albania, Montenegro, CEFTA and EU27. Both sectors together show slightly higher values of Competitiveness Index compared to sectors individually.



Graph 6 – Competitiveness Index for drinking water and beer sectors on markets of Croatia, Macedonia, Serbia and Turkey
Source: own calculation based on FTCB&H and UNCTAD data

Markets of Albania and Montenegro are markets with the highest values of Competitiveness Index, with values of Index in the only export category during the four years of period. All other markets show Index in first Index category, showing no competitive advantages achieved.
Graph 6 shows Competitiveness Index for Croatia, Macedonia, Serbia and Turkey in beer and drinking water sectors. For Macedonia and Serbia values of Competitiveness Index are dynamic in nature, for other export markets, Competitiveness Index values are showed in firs index category.

CONCLUSION

Two types of conclusions can be drawn from the results, one of them considers competitiveness of beer and water sector internationally, second of them considers the use of Competitiveness Index. For competitiveness, drinking water sector shows competitive advantages in markets of Albania and Montenegro – showing only export categories. Beer sector shows competitive advantages in market of Albania – showing only export categories. Both sectors combined show dynamic and low competitiveness level on all markets, excluding market Albania. Therefore, one can say that bot sectors, individually or as a group, are more competitive on less demanding markets suggesting competition based on price. As for the Competitiveness Index, it shows itself as a more rigorous measure of international competitiveness and provides answers to which sector achieved competitive advantage and on which product characteristic competitive advantage is based (price or quality). Downside of Competitiveness Index is that it requires graphical representation to be interpreted correctly, which implies the further development of index, probably developing fuzzy logic algorithms to determine competitive advantages based on Competitiveness Index.

REFERENCES

Abdul Malek, N. A., Shahzad, K., Takala, J., Bojnec, S., Papler, D., & Liu, Y. (2015). Analyzing Sustainable Competitive Advantage: Strategically Managing Resource Allocations to Achieve Operational Competitiveness. *Management and Production Engineering Review*, *6*(4).

Bahta, S., & Jooste, A. (2005). The effect of internationalization on the beef and maize sub-sectors: the relevance of revealed comparative advantage measures. *Agrekon, 44*(4), 452-464.

Balassa, B. (1965). Trade liberalization and "Revealed" Comparative Advantage. *The Manchester School*, *33*, 99-123 %M 110.1111/j.1467-9957.1965.tb00050.x.

Banterle, A., & Carraresi, L. (2007). Competitive performance analysis and European Union trade: The case of the prepared swine meat sector. *Food Economics - Acta Agriculturae Scandinavica, Section C, 4*(3), 159-172.

Banterle, A., Cavaliere, A., Carraresi, L., & Stranieri, S. (2014). Food SMEs Face Increasing Competition in the EU Market: Marketing Management Capability Is a Tool for Becoming a Price Maker. *Agribusiness, 30*(2), 113-131.

Bojnec, S., & Ferto, I. (2006). Competition and dynamics in trade patterns: Hungarian and Slovenian agri-food trade with the European Union's trading patterns. *Presented at the International Association of Agricultural Economists Conference, Gold Coast, Australia, August 12-18, 2006*.

Bojnec, S., & Ferto, I. (2007a). Determinants of competition in agro-food trade between Central European Countries and the European Union. *Presented at the joint IAAE-104th EAAE Seminar, Budapest, Hungary, September 6-8, 2007*.

Bojnec, S., & Ferto, I. (2007b). Trade advantages and integration of Croatian, Hungarian and Slovenian agro-food trade with the European Union. *Periodica Polytechnica Social and Management Sciences, 15*(1), 3.

Bojnec, S., & Ferto, I. (2009a). Agro-food trade competitiveness of Central European and Balkan countries. *Food Policy, 34*(5), 417-425.

Bojnec, S., & Ferto, I. (2009b). Determinants of agro-food trade competition of Central European countries with the European Union. *China Economic Review, 20*(2), 327-337.

Bojnec, S., & Ferto, I. (2014). Agri-food exports from European Union Member States using constant market share analysis. *Studies in Agricultural Economics, 116*(2), 82-86.

Bojnec, S., & Ferto, I. (2014). Export competitiveness of dairy products on global markets: The case of the European Union countries. *Journal of Dairy Science, 97*(10), 6151-6163.

Bojnec, S., & Ferto, I. (2015a). Agri-Food Export Competitiveness in European Union Countries. *JCMS: Journal of Common Market Studies, 53*(3), 476-492.

Bojnec, S., & Ferto, I. (2015b). Are new EU member states catching up with older ones on global agri-food markets? *Post-Communist Economies, 27*(2), 205-215.

Brander, J. A., & Spencer, B. J. (2015). Intra-industry trade with Bertrand and Cournot oligopoly: The role of endogenous horizontal product differentiation. *Research in Economics, 69*(2), 157-165.

Herciu, M. (2013). Measuring International Competitiveness of Romania by Using Porter's Diamond and Revealed Comparative Advantage. *Procedia Economics and Finance, 6*, 273-279.

Kocourek, A. (2015). Structural Changes in Comparative Advantages of the BRICS. *Procedia - Social and Behavioral Sciences, 172*, 10-17.

Marius-Razvan, S., & Camelia, S. (2015). Analysis of the Intra-Industry Trade for the Motor Vehicle Parts and Accessories Sector from Romania. *Procedia Economics and Finance, 22*, 343-352.

Nikolić, A. (2008). *Konkurentska sposobnost industrije mlijeka u Bosni i Hercegovini.* (Doctoral), Univerzitet u Sarajevu, Sarajevo, Bosna i Hercegovina.

Nikolić, A., Bajramović, S., Ognjenović, D., Lalić, D., & Uzunović, M. (2011). SEE trade liberalisation – new opportunity for B&H agrobusiness? *British Food Journal, 113*(1), 78-95.

Utkulu, U., & Seymen, D. (2004). Revealed comparative advantage and competitiveness: evidence for Turkey vis-a-vis the EU15. *Presented at the European Trade Study Group 6th Annual Conference, Nottingham, UK, September 2004*.

Uzunović, M. (2010). Konkurentske prednosti prehrambene industrije Bosne i Hercegovine. *Master rad, Poljoprivredno-prehrambeni fakultet, Sarajevo.*

Yoshida, Y. (2013). Intra-industry trade, fragmentation and export margins: An empirical examination of sub-regional international trade. *The North American Journal of Economics and Finance, 24*, 125-138.

CHANGES OF PRODUCTION, CONSUMPTION AND FOREIGN TRADE IN TURKEY'S AGROCHEMICALS SECTOR*

M.Cagla Ormeci Kart[1], Sule Isin[1]

Original scientific paper

Summary

Agrochemicals, including pesticides, are considered a critical aid to improving agricultural production and prevention of crop losses pre and post-harvest. This study aims to make an evaluation of the changes in production, usage and the trade of pesticides in Turkey. The principal material of the study comprises data and reports of the Republic of Turkey Ministry of Food Agriculture and Livestock, Food and Agriculture Organization (FAO) and United Nations Commodity Trade (UN Comtrade). Pesticides classified under code 38.08 in the International Convention on the Harmonized Commodity Description and Coding System and include hazardous pesticides, insecticides, fungicides, herbicides, disinfectants and other. In addition, this study benefited from previous similar studies. The relevant data are presented in tables. According to the final data Turkey's pesticides production, consumption and foreign trade shows an increment. Turkey agrochemicals market is smaller than EU. Turkey's pesticide usage is 400-700 gram per hectare but in some regions is equal with the world's most intensive pesticide-using regions.

Key words: *export, herbicide, import, insecticide, pesticide*

INTRODUCTION

Today 1600 of diseases, 10 000 of pests, 30 000 herbicides and other noxious (viruses, bacteria, and nematodes) cause significant damage to the plants and crops. These noxious have destroyed averagely one of the third of crops according to the surveys. This noxious, which causes 60-70% or even nearly 100% loss of crops, is an important issue of mankind to combat pests, diseases and weed, to reduce and even to prevent damage (Anonim, 2001; Akgün, 2001). According to statistics, crop losses, which are caused by diseases, pests and weeds in the world range from 27.5 to 60 billion USD. According to the FAO records, only 23 million tons of grain and disease-related losses are due to the annual demand of 150 million people (Anonim, 2001). Agrochemicals, including pesticides, are considered a critical aid to improving agricultural production and prevention of subjected crop losses pre and post-harvest.

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Ege University, Faculty of Agriculture, Department of Agriculture Economics, 35100 Bornova, İzmir, Turkey

Along with the increasing use of pesticides, there is also an increasing awareness of the effect of pesticides on human health and the environment in general. In the global context the Food and Agriculture Organization of the United Nations has developed the International Code of Conduct on the distribution and use of pesticides (FAO, 1986; Rahman *et al*., 1995).

Hassall (1982) bases this short summary of the history of pesticide use on a review. The use of inorganic chemicals to control insects possibly dates back to classical Greece and Rome. The Chinese were employing moderate amounts of arsenicals as insecticides by the sixteenth century and not long afterwards, nicotine was used in the form of tobacco extracts. By the nineteenth century, both pyrethrum and soap had been used for insect control, and a combined wash of tobacco, sulphur, and lime to combat insects and fungi. The middle of the nineteenth century marked the beginning of the first systematic scientific studies into the use of chemicals for crop protection. Work on arsenic compounds led to the introduction in 1867 of Paris green, an impure form of copper arsenide. It was used in the USA to check the spread of the Colorado beetle and by 1900; its use was so widespread that it led to the introduction of what was probably the first pesticide legislation in the world. In 1896, a French grape grower, applying Bordeaux mixture to his vines, observed that the leaves of yellow charlock growing nearby turned black. This chance observation demonstrated the possibility of chemical weed control, and shortly afterwards it was found that iron sulphate, when sprayed on to a mixture of cereal and weeds killed the weeds without damaging the crop. Within a decade, several other inorganic substances have been shown to act selectively at appropriate concentrations.

Another important landmark was the introduction of the first organomercury seed dressings in 1913 in Germany. In the years between the First and Second World Wars, both the number and the complexity for crop protection increased. Tar oil was, and still is, used to control the eggs of aphids on dormant trees. During the Second World War, the insecticidal potential of DDT was discovered in Switzerland and insecticidal organophosphorus compounds were developed in Germany. At about the same time, work was in progress in the United Kingdom that was to lead to the commercial production of herbicides of the phonoxyalkanoaic acid group. In the period from 1950-1955, urea derivatives were developed as herbicides in the USA, the fungicides captan and glyodin appeared, and malathion was introduced. Between 1955 and 1960, other new products included herbicidal triazines and quaternary ammonium herbicides. Dichlobenil, trifluarin, and bromoxynil were described between 1960 and 1965 the systemic fungicide benomyl in 1968. The leaf-acting herbicide glyphosate was introduced soon afterwards. During the 1970’s and 1980s, many new pesticides were introduced. They have been based on a more thorough understanding of biological /biochemical mechanism, and they are often more effective at lower doses than the older pesticides. Because of a better knowledge of host-pest interactions, a new approach to the design of pesticides is now being developed, as well as new strategies for formulations, and new methods of application. These developments provide an opportunity to reduce the risk of pesticide poisoning (WHO, 1990).

FAO (1986) defined a pesticides as any substance or mixture of substances intended for preventing, destroying, or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during, or otherwise interfering with, the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products, or animal feedstuffs, or which may be administered to animals for the control of insects, arachnids, or other pests is or on their bodies. The term includes substances intended for use as a plant-growth regulator, defoliant, desiccant, fruit-thinning agent, or an agent for preventing the premature fall of fruit, and substances applied to crops either before or after harvest to prevent deterioration during storage or transport. The Codex Alimentarius Commission (Codex, 1984) and the Council of Europe (1984) have adopted similar definitions. In each case, the term excludes fertilizers, plant and animal nutrients, food additives, and animal drugs. This study aims to make an evaluation of the changes in production, usage and the trade of pesticides in Turkey

MATERIALS AND METHODS

The principal material of the study comprises data and reports of the Republic of Turkey Ministry of Food Agriculture and Livestock, Food and Agriculture Organization (FAO) and United Nations Commodity Trade (UN Comtrade). In addition, this study benefited from previous similar studies. The relevant data are presented in tables. The biggest constraints is that the lack of statistics. No detailed data available according to the pesticides type such as herbicides, insecticides or fungicides.

RESULTS AND DISCUSSION

Pesticide use in the world

The most insecticide using countries between the years 1990-2014 was listed in Table 1. When we look at average usage quantities USA is the biggest insecticide user countries followed by Brazil, Mexico, India, Japan, Argentina, Turkey, Italy, Spain, Columbia, Korea, Ukraine, Thailand, Bolivia and Paraguay. Brazil and Mexico insecticide usage show a significant linear increase between the years 1994-2014. Insecticides use of other countries shows fluctuations. The most herbicide using countries between the years 1990-2014 was listed in Table 2. When we look at average usage quantities, USA is the biggest insecticide user countries followed by Argentina, Brazil, Ukraine, Thailand, Mexico, Columbia, Bolivia, Japan, Spain, Italy, Turkey, Korea and India.

The worldwide consumption of pesticides is about two million tonnes per year; out of which 45 % is used by Europe alone, 25% is consumed in the USA and 25% in the rest of the world. India's share is just 3.75% (De *et al*., 2014). According to the current statistics, the increase in world pesticide consumption seems to have slowed down in recent years. Pesticide regulation in the world has encouraged fewer, yet less toxic, new chemical pesticides.

The three most commonly used pesticides are HCH (only gamma-HCH is allowed), DDT, and malathion, and these account for about 70% of the total pesticide consumption. Despite development of newer pesticide, these pesticides still remain the choice of small farmers because they are cost-effective, easily available, and display a wide spectrum of bioactivity (De *et al.*, 2014). As shown in Figure 1, herbicides are ranked first in agrochemicals with a share of 50% in the world pesticide usage. Fungicides and bactericides follow by 24%; insecticides by 15%. These three groups cover nearly 90% of the use. Other pesticide groups have a share of 11%. On the contrary, the consumption of herbicides in Turkey is probably low (%20), because weed control is mainly done manually by hand or machinery.

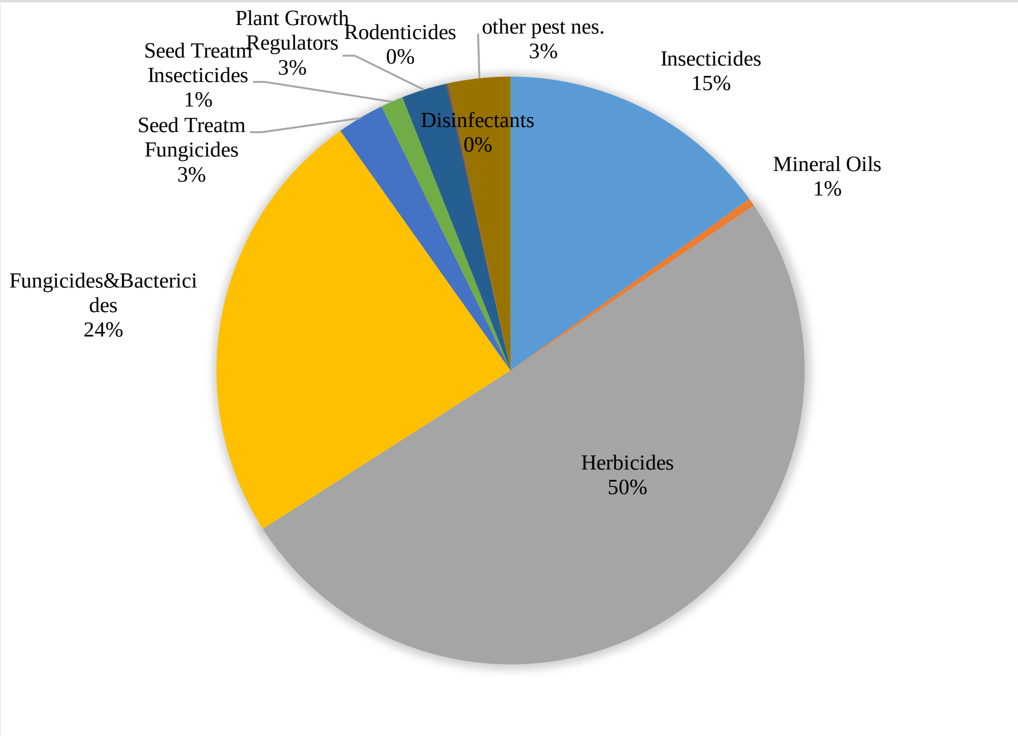


Fig 1. Distribution of world pesticide use according to the chemical type in 2014 (FAOSTAT, 2017)

Table 1. Insecticide use in the world between the years 1990-2014 (tonnes of active ingredients)

Country	1990	1995	2000	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	Average
USA	86182.5	104326.2	100243.8	75749.9	74842.7	78471.4	NA	NA	NA	NA	NA	NA	NA	86636.07
Brazil	18388.0	NA	28382.0	43763.0	45435.0	57421.0	65642.0	61180.0	66471.0	60614.0	57170.0	70424.0	NA	52262.73
Mexico	NA	NA	18878.0	19188.0	19750.0	27196.0	25155.0	26024.0	26671.0	30823.0	37501.0	37455.0	32406.0	27367.91
India	57945.0	40045.0	NA	21783.0	16913.0	14617.6	3278.3	14810.2	20618.8	NA	NA	NA	NA	23751.37
Japan	NA	NA	27292.3	22679.6	24031.8	22549.0	21146.4	23403.1	20535.0	18847.5	19066.3	17547.8	18030.2	21375.36
Argentina	NA	6188.0	NA	NA	18684.0	23659.0	18695.0	15857.0	26519.0	23240.0	NA	NA	7919.3	17595.16
Turkey	17652.0	14639.0	13910.0	16032.0	8530.0	22012.0	9988.0	11447.0	8216.0	7182.0	8123.0	8599.0	9099.0	11956.08
Italy	12074.0	12233.0	8859.0	12838.0	12470.0	13567.0	13523.0	10930.0	10834.0	11795.0	10413.0	8713.0	9099.0	11334.46
Spain	9194.0	9538.0	10470.0	14144.0	13438.0	12740.0	13294.0	12624.0	14335.0	8146.0	7704.0	6909.0	7609.0	10780.38
Colombia	4006.0	4240.0	12743.0	36456.6	12121.7	11714.5	8198.9	7457.5	8717.6	12198.0	5115.8	9401.4	7688.5	10773.81
Korea	NA	NA	765.0	NA	NA	NA	NA	NA	NA	NA	20329.4	6943.7	NA	9346.03
Ukraine	NA	NA	NA	3331.5	4508.0	5675.8	8522.3	5903.4	8905.8	11150.0	12406.2	14703.1	12851.8	8795.79
Thailand	9332.0	8892.0	8625.0	8734.0	8334.0	8568.0	9960.0	8810.0	7414.0	6634.0	5047.0	5991.0	6620.0	7920.08
Bolivia	1038.0	NA	NA	5535.0	5852.9	6059.0	5947.4	7281.1	9859.0	9191.0	9386.0	9390.3	8914.0	7140.29
Paraguay	NA	6827.0	5278.0	6650.0	5187.0	8773.0	9471.0	8112.0	9995.0	10671.0	4770.0	1675.0	6838.0	7020.58

Source: FAOSTAT, 2017

Table 2. Top herbicide using countries between the years 1990-2014 (tonnes of active ingredients)

Area	1990	1995	2000	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	Average
USA	206384.36	209105.91	195951.74	190962.23	184611.94	200487.66								197917.31
Argentina		24863.00			143635.00	180337.00	199376.00	135077.00	227185.00	212337.00			194257.30	164633.41
Brazil	22903.00		81862.00	136853.00	144986.00	189101.00	185665.00	202554.00	189537.00	188745.00	214201.00	239657.00		163278.55
Ukraine				14843.20	18818.10	22797.00	35254.40	21647.90	40977.90	50678.00	62186.60	52492.60	49952.90	36964.86
Thailand		11935.00	10950.00	31116.00	29463.00	50174.00	47646.00	53615.00	51900.00	67608.00	60231.00	1220.00	11091.00	35579.08
Mexico			19760.00	32823.00	32344.00	36587.00	32582.00	32019.00	31878.00	34735.00	37684.00	31195.00	26392.00	31636.27
Colombia	6573.00	8322.00	27139.00	44968.27	60994.81	38795.50	28006.61	21543.68	17587.14	23882.00	21167.90	30037.56	24917.20	27225.74
Bolivia	1128.00			11828.80	12356.00	14138.90	14374.80	17161.30	17242.00	19713.00	23645.00	28942.60	25070.35	16872.80
Japan			11331.70	12158.10	11946.70	12031.60	11857.20	12804.70	12166.70	11414.30	11563.60	11979.50	12349.50	11963.96
Spain	13395.00	6326.00	9942.00	10216.00	10577.00	10973.00	10388.00	8781.00	10060.00	13835.00	13985.00	14719.00	14909.00	11392.77
Italy	9602.00	9779.00	9432.00	9191.00	8721.00	9180.00	8435.00	7637.00	9934.00	8320.00	7981.00	7690.00	7585.00	8729.77
Turkey	6258.00	4842.00	6960.00	11716.00	8170.00	6668.65	6176.51	5960.85	7451.59	7407.00	7351.00	7336.00	7794.00	7237.82
Korea	5509.00	5817.00	5822.00	6189.00	5814.00	5946.00	6073.00	5909.00	5224.00	5180.00	4432.00	4479.00	5536.00	5533.08
India	5821.00	6120.00		6959.00	6304.00	4121.66	3574.67	4219.98	6334.98					5431.91

Source: FAOSTAT, 2017

Developments in pesticides trade

Global developments in pesticides trade are presented in Table 3 and Table 4. The top insecticide importer countries between the years 1990-2014 was listed in Table 3. When we look at average import value France is the biggest followed by Brazil, Germany, Canada, United Kingdom, Belgium, United States of America, Italy, Spain, Netherlands, Poland, Thailand, Argentina, India, Mexico, Ukraine, Australia, Viet Nam, China and Russia Federation. India and China insecticide import value show a significant linear increase between the years 1994-2014. Insecticide import value of other countries also shows an increasing trend beside some fluctuations.

The biggest insecticide exporters' countries between the years 1990-2014 was listed in Table 4. When we look at average export value, Germany is the biggest insecticide exporter country followed by France, USA, China, Belgium, United Kingdom, India, Switzerland, Netherlands, Spain, Italy, Israel, Argentina, Brazil, Colombia, Malaysia, Singapore, Indonesia, Hungary and Republic of Korea. According to the current statistics, the increase in world pesticide export seems to have slowed down in recent years.

Table 3. Pesticide import value in the world between the years 1990-2014 (1000 $)

	1990	1995	2000	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	Average
France	1278389	1479531	1387807	1774133	1462328	1733856.20	2119220.61	2108156.48	1803027.11	2239470.27	2229338.71	2469921.48	2705754.48	1906994.87
Brazil	41606	105089	265809	654790	516941	835127.09	1267647.51	1301086.50	1529949.84	1958809.31	2245806.26	2999750.88	3464130.61	1322041.77
Germany	585990	590203	600541	1000303	975375	1193827	1551063	1450661	1291753.61	1767267.80	1775634.61	1853297.66	1977005.76	1277917.11
Canada	292840	506592	518269	738476	860775.60	928712.68	1109533.52	1205565.21	1104852.93	1057298.72	1195855.36	1546552.57	1556135.43	970881.46
UK	314323	491886.80	431685.20	672379.50	642086	847551.26	988632.46	NA	870593.87	982838.39	976602.50	948559.50	1120095.49	773936.16
Belgium	NA	NA	275649	500763	547171	732903.97	880458.76	883515.18	743280.07	907166.43	883997.32	979621.35	940351.85	752261.63
USA	217927	350396	471168	724512.63	655367	730669.61	828459.86	775089.19	772284.71	839328.16	886581.65	997604.82	1074804.40	717245.62
İtaly	293494	401040	338970	582209	603081	752591.85	831475.48	818015.95	786997.80	923278.21	906396.08	884661.07	966365.91	699121.26
Spain	202480	295134	323124.20	474110.80	450229.40	585211.99	743557.84	722260.60	680976.79	898662.85	821271.62	904719.94	976470.81	621400.83
Netherlands	319284	340462	253735	435957	496295	590186.16	580593.56	537199.18	592783.10	830997.50	739982.18	822287.60	809477.77	565326.16
Poland	80000	139318	206591	432375.40	473459.80	517557.24	776157.25	552601.07	592821.97	735662.24	697047.36	777954.49	889876.67	528570.96
Thailand	130495	150000	194154	262885.21	344326.05	419949.50	557900.56	466615.96	513651.36	684388.64	626269.82	799172.41	696912.43	449747.76
Argentina	68052	207170	174651	225710.92	222655.40	360291.26	453857.29	297644.62	530851.63	654293.38	676964.38	755907.93	709454.51	410577.26
İndia	16203	39987	49212	171121	178006	193891.84	341507.67	439025.54	629274.21	730999.62	724627.63	792573.27	990318.95	407442.13
Mexico	45773	145769	230942.10	340481	365890	406643.91	467740.88	421688.91	492287.67	536463.28	545219.28	598650.18	694907.74	407112.07
Ukraine	NA	114300	62101	173675.30	201859	290021.02	414432.89	270567.17	449812.53	650731.26	754596.81	769455.22	608350.09	396658.52
Australia	42794	95625	198139	284037	284262	301666.08	414049.32	354301.88	519104.49	623339.04	655134.44	669116.70	691338.79	394839.06
Vietnam	9043	114300	62101	173675.30	201859	290021.02	414432.89	270567.17	449812.53	664909.79	699788.26	786220.94	829497.16	382017.54
China	177776	159743	192227	182214	212814	231460.08	296329.94	334291.26	421353.22	488706.92	592237.03	692919.44	770625.96	365592.14
Russia	NA	163981	102345	194281.04	178787.14	336675.88	528803.19	387026.11	327562.55	452630.92	497762.27	556928.44	600498.12	360606.81
Turkey	60919	91487	97744	156216	199154.79	224357.89	254202.37	208501.58	277032.21	360846.57	331557.11	324263.03	329759.28	224310.83

Source: FAOSTAT, 2017.

Table 4. Herbicide import value in the world between the years 1990-2014 (1000 $)

	1990	1995	2000	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	Average
Germany	1542408.00	1750194.00	1858538.00	1908079.00	2355308.00	2152983.00	3354668.00	3048147.00	2982991.37	3760947.02	3670432.88	4146483.69	4325714.87	2835145.76
France	1034945.00	1515493.00	1700933.00	2566852.00	2477606.00	2579473.40	3074810.47	2721871.13	2752830.41	3474480.91	3522982.94	3784790.17	4410101.05	2739782.27
USA	1157245.00	1320296.00	1492417.00	1621195.30	1906288.00	2211715.91	2475963.47	2405949.06	2677620.65	2850076.92	3304017.59	3914188.03	4194663.78	2425510.52
China	68453.00	233987.00	462953.00	1400079.00	1039620.00	1349671.47	2015620.77	1415556.03	1769683.59	2418751.86	2852134.42	3744214.08	4146984.44	1762900.67
Belgium	NA	NA	373953.00	1154034.00	1304887.00	1512785.40	2196480.29	1807012.24	1524530.25	1850552.56	1788660.54	2153457.57	2055873.91	1611111.52
UK	975514.00	1130583.60	1065537.40	1135607.10	1020552.90	1146156.18	1320339.76	NA	1291828.01	1528365.24	1488181.94	1601303.20	1471730.53	1264641.66
India	58549.00	162201.00	270631.00	633006.00	635131.00	709701.84	1017954.78	1037928.81	1140234.05	1380427.45	1660847.83	2133216.91	1939991.05	983063.13
Switzerland	571659.00	722437.00	556336.00	755005.00	644567.00	837880.06	1015092.36	817030.24	945216.75	1070945.60	971316.57	1052063.78	1117184.29	852056.43
Netherlands	427877.00	472144.00	287649.00	661487.00	623290.00	661195.09	740498.99	645142.33	739820.64	940272.95	898741.50	896701.49	868351.01	681782.38
Spain	45310.00	100977.00	249049.90	514093.10	443949.90	522618.46	836375.05	673255.98	752428.54	1023460.59	1144618.01	1209164.83	1293073.33	677567.28
Italy	206064.00	247459.00	327301.00	533553.00	512257.00	600304.63	661864.86	614246.42	658120.74	774156.58	717385.74	767099.32	770637.13	568496.11
Israel	44309.00	108554.00	143013.00	474304.00	493999.00	NA	811283.00	565770.00	682579.00	666359.00	748769.00	754918.00	963394.00	538104.25
Argentina	38526.00	59864.00	109290.00	248938.34	229691.70	316003.31	581800.01	378598.70	413412.45	406539.67	408612.52	555907.70	554480.46	330897.30
Brazil	61238.00	127546.00	146299.00	233968.00	242235.00	366175.30	432034.00	331669.18	422939.29	471728.16	428697.73	364206.51	325255.81	304153.23
Colombia	46760.00	141169.00	184443.00	195638.00	178312.00	204058.84	209347.34	246131.86	229374.08	229865.08	282124.09	441976.05	392564.40	229366.44
Malaysia	25828.00	57544.00	60212.00	89915.01	128479.72	182818.47	158991.40	215915.87	236403.14	226880.42	303820.78	356682.97	344793.98	183714.29
Singapore	33816.00	65963.00	149043.00	176368.42	136464.61	147878.52	217199.82	161129.96	169072.13	193757.08	207096.75	249966.02	371481.10	175325.88
Indonesia	25518.00	13574.00	70389.00	67038.00	89889.00	110158.63	135414.33	163436.83	201050.92	247434.47	249577.49	317445.09	300839.90	153212.74
Hungary	72483.00	67692.00	51410.00	63230.00	58869.00	131300.00	188888.00	171885.00	168739.00	216314.00	220342.00	245847.25	311559.79	151427.62
Republic of Korea	7392.00	18523.00	23225.00	59431.00	75139.00	91302.43	128043.18	143488.19	152611.47	194147.46	239369.45	272871.44	342721.15	134481.91
Turkey	4052.00	19258.00	23090.00	27859.00	33635.71	48465.66	63382.53	56698.67	64342.07	72009.24	76042.45	82138.78	93690.95	51128.08

Source: FAOSTAT, 2017.

Pesticide use in Turkey

The decrease in world pesticide consumption has also shown itself in Turkey. Table 5 shows the changes in Turkey's pesticide usage between the years 2006-2015. According to the Table 3 pesticide use in Turkey shows 14 per cent decrease in the last 10 years although some fluctuations. The decrease is very apparent on fungicides and other pesticides (plant growth regulator etc.) (Table 5). Turkey pesticide use is about 30 000 metric tons. Average consumption is 0.5 kg per hectare. This quantity is comparably low to averages in developed countries. However, consumption of pesticide is very heterogeneous in Turkey. More than sixty-five percent of pesticides are used in the Aegean and Mediterranean regions (SEEP, 2010).

Table 5. Pesticide use in Turkey according to the chemical type between the years 2006-2015 (tonnes of active ingredients)

Year	Insecticides	Index	Fungicides	Index	Herbicides	Index	Acaricides	Index	Rodenticides	Index	Other	Index
2006	7628	100.00	19900	100.00	6956	100.00	902	100.00	3	100.00	9987	100.00
2007	21046	275.90	16707	83.95	6669	95.87	966	107.10	51	1700.00	3277	32.81
2008	9251	121.28	17863	89.76	6177	88.80	737	81.71	351	11700.00	5613	56.20
2009	9914	129.97	17396	87.42	5961	85.70	1533	169.96	78	2600.00	2302	23.05
2010	7176	94.07	17546	88.17	7452	107.13	1040	115.30	147	4900.00	5344	53.51
2011	6120	80.23	18124	91.08	7407	106.48	1062	117.74	421	14033.33	6978	69.87
2012	7264	95.23	15525	78.02	7351	105.68	859	95.23	247	8233.33	8766	87.77
2013	7741	101.48	16248	81.65	7336	105.46	858	95.12	129	4300.00	7128	71.37
2014	7586	99.45	16674	83.79	7794	112.05	1513	167.74	149	4966.67	6007	60.15
2015	8117	106.41	15984	80.32	7825	112.49	1576	174.72	197	6566.67	5327	53.34

Source: Ministry of Food, Agriculture and Livestock

Pesticide industry in Turkey

The agrochemicals industry in Turkey started to establish in 1951. Especially in 1957, the law introduced the necessity of all drugs to be produced in international standards. Since 1985, researches on pest management have started to be carried out in Turkey and since 1991, it has been carried out by the General Directorate of Agricultural Researches and Projects (Dağ *et al*., 2000). Table 6 shows the production quantity of pesticides in Turkey in some years between the years 1995-2015. Herbicide and insecticides show an increase trend while the others started to produce less. In 1995 Turkey insecticides production was 8938 ton and in 2015 it was doubled and reach to 18351.70 metric ton. A remarkable increase also occurred in herbicide production in 1995 herbicide production was7267 ton and has quadrupling by reaching 30245 ton. According to the data of 2005, there are 165 active pesticide firms operating in Turkey and over 3000 licensed medicines are available. According to the records, although the capacity of the enterprises is high, only 5-6% of the total capacity can be used. 97% of the total capacity belongs to the domestic firm whereas the remaining 3% belongs to the foreign firms (Anonyms, 2008).

Table 6. Turkey pesticides manufacturing (tonnes of active ingredients)

	Insecticides	Index	Herbicides	Index	Fungicides & Bactericides	Index	Acaricide	Index
1995	8938.00	100.00	7267.00	100.00	3749.00	100.00	776.00	100.00
1996	14089.00	157.63	6182.00	85.07	10928.00	291.49	497.00	64.05
1997	9755.00	109.14	6954.00	95.69	8505.00	226.86	512.00	65.98
1998	11734.00	131.28	4222.00	58.10	5798.00	154.65	453.00	58.38
1999	9516.00	106.47	5663.00	77.93	5650.00	150.71	255.00	32.86
2000	10107.93	113.09	5349.78	73.62	6000.01	160.04	557.99	71.91
2001	9054.91	101.31	5217.66	71.80	4651.77	124.08	205.13	26.43
2002	11346.27	126.94	5010.17	68.94	6812.27	181.71	262.26	33.80
2003	10203.50	114.16	8261.44	113.68	9134.11	243.64	159.24	20.52
2004	11017.09	123.26	6418.78	88.33	4138.27	110.38	389.48	50.19
2011	13969.19	156.29	42267.31	581.63	1897.59	50.62		
2012	14368.30	160.76	45676.76	628.55	3348.72	89.32		
2013	14262.69	159.57	27302.41	375.70	2241.74	59.80		
2014	15198.55	170.04	34120.04	469.52	2173.54	57.98		
2015	18351.70	205.32	30245.01	416.20	2016.84	53.80		

Source: Anonyms, 2008; TURKSTAT, Industrial production statistics, 2017.

Pesticides trade in Turkey

In Turkey, the rate of dependency on agriculture chemical is very high. However, in recent years important developments have been recorded regarding the production of effective substances. Turkish farmers prefer chemical management and an average of 30-35 thousand tons of pesticide are produced annually. Its monetary value is about $250 million and about 80% of the effective substances are imported from abroad (Anonyms, 2008). Table 7 shows Turkey's pesticides export and import value between the years 2000 and 2016. Pesticide export and import value of Turkey show similar trends. Both of them increase very rapidly in the last 15 years. In 2016, pesticide export value of Turkey reached $88.78 million and import value was $356.28.

Agrochemical trade of Turkey has a very small share in the total export and import. Pesticide export value of Turkey shows a linear increase in the last sixteen years but the share is almost the same, which varies between 0.04 and 0.08%. Pesticide import value of Turkey shows similarly a linear increase but the share in the total import value changes between 0.13 and 0.18 % in the last sixteen years.

One of the most commonly used of trade indicator is the normalized trade balance, which is the ratio of the trade balance to the total value of trade (exports plus imports). This is an indicator with a variation range limited between - 1 and 1, which is equal to zero when exports are equal to imports and reaches the extreme values in those limited cases in which exports or imports are non-existent. Low and negative normalized balances are recorded for commodities in which the national production is poor competitive in both foreign and domestic markets (Lelio Iapadre, 2001). The normalized trade balance of pesticide between the years 2000 and 2016 shows always a negative sign. Even in some movement, Turkish pesticide industry is very poor in foreign market. Nevertheless the target of the Turkish chemical industry is to export 110 million dollars and import 700 million dollars in the sub-sector of agrochemicals in 2018; in 2023, exports of 179 million dollars were set at $ 1.13 billion (Anonyms, 2015).

Table 7. Evaluation of Turkey's pesticides export and import (1000 $)

Year	Total export value (1000 $)	Pesticide export value (1000$)	Index (2000=100)	Share 0f total	Total import value (1000 $)	Pesticide import value (1000 $)	Index (2000=100)	Share of total	The normalized trade balance (X – M) / (X + M)
2000	27774906.05	23089.89	100	0.08	54502820.50	97744.09	100	0.18	-0.62
2005	73476408.14	27858.5	120.65	0.04	116774150.91	156215.67	159.82	0.13	-0.7
2006	85534676.00	33635.71	145.67	0.04	139576174.00	199154.79	203.75	0.14	-0.71
2007	107271749.90	48465.66	209.9	0.05	170062714.50	224357.89	229.54	0.13	-0.64
2008	132027195.63	63382.53	274.5	0.05	201963574.11	254202.37	260.07	0.13	-0.6
2009	102142612.60	56712.46	245.62	0.06	140928421.21	208501.58	213.31	0.15	-0.57
2010	113883219.18	64311.22	278.53	0.06	185544331.85	277032.21	283.43	0.15	-0.62
2011	134906868.83	72009.24	311.86	0.05	240841676.27	360846.57	369.17	0.15	-0.67
2012	152461736.56	75946.1	328.91	0.05	236545140.91	331557.11	339.21	0.14	-0.63
2013	151802637.09	82138.78	355.73	0.05	251661250.11	324263.03	331.75	0.13	-0.6
2014	157610157.69	93960.95	406.94	0.06	242177117.07	329759.28	337.37	0.14	-0.56
2015	143838871.43	94796.69	410.55	0.07	207234358.62	362902.92	371.28	0.18	-0.59
2016	142529583.81	88781.83	384.51	0.06	198618235.05	356287.58	364.51	0.18	-0.6

Source: TURKSTAT, Foreign Trade Statistics, 2017.

CONCLUSION

In summary, the world average production of agrochemicals is around 2.8 million tons, which is estimated to be about 30 billion US dollars. Results show that the fastest growth in pesticide demand is in developing countries in Eastern Europe, South America and Asia. Growing production along with improved farming techniques will drive demand in Eastern Europe.

Tilman (2002) highlighted that the development in the control of weedy competitors of crops, crop diseases and pathogens, and herbivores could significantly increase yields which means pesticides are generally profitable in agriculture; but sometimes their use does not always decrease crop losses (Pimentel, 2005). This results show us that pesticide industry are economically important in two ways. First, it is important in commercially as a traded good and secondly it is important in terms of human health and environment. Unconscious and uncontrolled use of pesticides may create resistance in pests and damage human health, and environment with the residuals in the long-term should not be ignored (Armagan and Ozden, 2015). Many research determined negative effects of pesticides such as acute poisonings, cancer, residues in food, domestic animal poisonings, contaminated products, destruction of beneficial natural predators, pesticide resistance, and reduction in pollination, fishery losses, ground and surface water contamination (Pimentel *et al.*, 1992; Rahman *et al.* 1995; Ecobichon, 2001; Gunier *et al.*, 2001; Tilman *et al.*, 2002, Gunnell and Eddleston, 2003; Pimentel, 2005; Sekiyama *et al.*, 2007; Yürekli Yüksel and Canik, 2011).

Currently, Turkish agrochemical sector has a weak position in the global market but the target of the Turkish chemical industry is to export 110 million dollars and import 700 million dollars in the sub-sector of agrochemicals in 2018; in 2023, exports of 179 million dollars were set at $ 1.13 billion (Anonyms, 2015). To reach these targets, Turkish agrochemical manufacturer should take into consideration the increasing demand in Eastern Europe and biological pesticides. Biological pesticides are environmentally appealing because they occur naturally. Biological pesticides currently have only a small share of the total pesticide market but it is expected that it will become more widespread in the direction of increasing consumer demand.

BIBLIOGRAPHY

A. De *et al.*, Targeted Delivery of Pesticides Using Biodegradable Polymeric Nanoparticles, Springer Briefs in Molecular Science, DOI: 10.1007/978-81-322-1689-6_2, The Author(s) 2014.

Akgün, C. 2001. Tarım İlaçları Sektör Araştırması, İstanbul Ticaret Odası, Dış Ticaret Araştırma Servisi, 40 sayfa, Istanbul.

Anonim, 2001; Sekizinci Kalkınma Planı Kimya Sanayi Özel İhtisas Komisyonu Raporu Tarim İlaçlari Alt Komisyonu Raporu.

Anonim, 2008. Dokuzuncu Kalkınma Planı Gübre-Tarım İlaçları Çalışma Grubu Raporu. http://www.kalkinma.gov.tr/Lists/zel%20htisas%20Komisyonu%20Raporlar/Attachments/33/oik704.pdf

Anonim, 2015. Onuncu Kalkınma Planı 2014-2018 KİMYA ÇALIŞMA GRUBU RAPORU http://www.kalkinma.gov.tr/Lists/zel%20htisas%20Komisyonu%20Raporlar/Attachments/259/Kimya%20%C3%87al%C4%B1%C5%9Fma%20Grubu%20Raporu.pdf

Armagan, G., Ozden, A. 2015. Food Production and Pesticide Use: A Dilemma in Agriculture. Fresenius Environmental Bulletin, 24(12): 4364-4368.

Codex (1984). Guide to Codex recommendations concerning pesticide residues. Part 1. General notes and guidelines. The Hague, Codex Alimentarius Commission (Report CAC/PRI 1984).

Council of Europe (1984). Pesticides. Advice and recommendations to be used by national and other authorities as well as manufacturers concerned with the registration of agricultural and non-agricultural pesticides. 6th edition, Strasbourg, l2Opp.

Ecobichon, D. J. 2001. Pesticide use in developing countries. Toxicology 160 (2001) 27–33.

FAOSTAT, 2017. Food and Agricultural Organization of the United Nations Statistics Database.

Food and Agricultural Organization of the United Nations, International Code of Conduct of the Distribution and Uses of Pesticides, FAO, Rome, 31 1986.

Gunier R., Harnly M., Reynolds P., Hertz A., Von Behren J. Agricultural pesticide use in California: pesticide prioritization, use densities, and population distributions for a childhood cancer study. Environ Health Perspect. 2001;109:1071–1078.

Gunnell, D., Eddleston, M. 2003. Suicide by intentional ingestion of pesticides: a continuing tragedy in developing countries. International Journal of Epidemiology 2003;32:902–909.

Hassall, K. A. 1982. The Chemistry of Pesticides. Wiley, 8 Eyl 1982 – 400p.

Lelio Iapadre, P. 2001. Measuring International Specialization. IAER: MAY 2001, VOL. 7, NO. 2.

Pimentel, D., H. Acquay, M. Biltonen, P. Rice, M. Silva, J. Nelson, V. Lipner, S. Giordano, A. Horowitz and M. D'Amore Environmental and Economic Costs of Pesticide Use; BioScience, Vol. 42, No. 10 (Nov., 1992), pp. 750-760.

Pimentel, D. 2005. Environmental and Economic Costs Of The Application Of Pesticides Primarily In The United States?. Environment, Development and Sustainability (2005) 7: 229–252 Springer 2005. DOI 10.1007/s10668-005-7314-2.

Rahman, M. S., Malek, M. A., Matin, M. A. 1995. Trend of pesticide usage in Bangladesh. The Science of the Total Environment, 159 (1995): 33-39.

SEEP, 2010. Pesticide use in cotton in Australia, Brazil, India, Turkey and the USA. https://www.icac.org/seep/documents/reports/2010_interpretative_summary.pdf

Sekiyama M1, Tanaka M., Gunawan B., Abdoellah O., Watanabe C. 2007. Pesticide usage and its association with health symptoms among farmers in rural villages in West Java, Indonesia. Environ Sci. 2007;14 Suppl:23-33.

Tilman, D., Kenneth G. Cassman, Pamela A. Matson, Rosamond Naylor & Stephen Polasky. 2002. Agricultural sustainability and intensive production practices. Nature, 418: 671-677.

WHO, 1990. World Health Organization, 1990. Public Health Impact of Pesticides Used in Agriculture. WHO, Geneva.

Yürekli Yüksel, N., Canik, F., 2011. Türkiye'de Tarım İlaçları Kullanımı. TEPGE BAKIŞ 2011 / ISSN: 1303–8346 / Nüsha: 3.

Dağ, S., V. T. Aykaç, A. Gündüz, M. Kantarcı ve N. Şişman. 2000. Türkiye'de Tarım Đlaçları Endüstrisi ve Geleceği. Türkiye Ziraat Mühendisliği V. Teknik Kongresi, 17-21 Ocak 2000. TMMOB Ziraat Mühendisleri Odası, Ankara. 933-957.

UTICAJ PROTOKA DIZNI NA KVALITET I TROŠKOVE ZAŠTITE BILJA*

INFLUENCE OF THE FLOW OF NOZZLES ON THE QUALITY AND COSTS OF PLANT PROTECTION

Selim Škaljić[1], Nermin Rakita[1]

Original scientific paper

Rezime

U Bosni i Hercegovini (BiH) prisutna je pojava da farmeri koriste prskalice sa neispravnim diznama. Ta situacija se reflektuje na količinu i loš kvalitet aplikacije fitofarmaceutskog sredstva (FFS). Neispravne dizne pored kvaliteta utiču i na troškove zaštite bilja. Prema cijenama FFS u BiH prosječni trošak jednog hemijskog tretiranja herbicidima košta 64,37 KM/ha, fungicidima 79,20 KM/ha i insekticidima 18,80 KM/ha. Rukovođeni pojavom korištenja neispravnih dizni, iz maloprodaje odabrano je 5 brendova najzastupljenih dizni u BiH. Sve odabrane dizne bile su sa protokom 1,6 l/min (*0,4 galona na pritisku 3 bara*) i uglom prskanja 110 stepeni. Za ispitivanje korištena je traktorska nošena ratarska prskalica zapremine rezervoara 330 litara. Radni pritisak ispitivanja podešen je na 3 bara a brzina aplikacije FFS na 6 km/h. Mjerenje pokrivenosti i kvaliteta aplikacije FFS utvrđivano je pomoću vodeno-senzornih listića, koji su nakon aplikacije prirodno sušeni, fotografisani a potom podvrgnuti digitalnoj obradi analize slike. Prosječna pokrivenost FFS kretala se u rasponu od 9,78% do 22,46%. Izmjereni protok dizni kretao od 1140 ml/min do 1700 ml/min. Prosječna veličina kapljica kretala se od 67,93 do 154,14 piksela. Na temelju dobivenih rezultata zaključeno je da protok dizni ima uticaj na pokrivenost lista, veličinu kapljica i troškove zaštite bilja.

Ključne riječi: *ispitivanje dizni, ratarska prskalica, fitofarmaceutska sredstva, kvalitet prskanja, veličina kapljica, troškovi zaštite biljaka.*

Summary

In Bosnia and Herzegovina (BiH) farmers are using farming sprayers with defective nozzles. This situation reflects on the quantity and poor quality of application of phytopharmaceutical products. Defective nozzles affect not just the quality but also the costs of crops protection. According to the prices of phytopharmaceutical products in BiH, the average costs of one chemical treatment with herbicides costs 64.37

---

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Poljoprivredno - prehrambeni fakultet Univerziteta u Sarajevu / *Faculty of Agricultural and Food Sciences University of Sarajevo.*

KM/ha, fungicides 79.2 KM/ha and insecticides 18.8 KM/ha. The awareness of use of defective nozzles motivated us to test randomly selected nozzles of 5 manufacturers available on the market in BiH and most popular in BiH. All the selected nozzles had the same flow of 1.6 l/min (0.4 gallons at the pressure 3 bar) and spraying angle of 110 degrees. A conventional 330 l farming sprayer connected to a tractor was used for the testing of the nozzles. The working pressure was set to 3 bars and the speed of application of pharmaceuticals to 6 km/h. The coverage and quality of the treatment was determined by the water-sensitive paper (WSP), which were left to dry naturally after the application, photographed and then subjected to digital processing and image analysis. The average application coverage ranged from 9.78% to 22.46%, with an average flow ranging from 1140 ml/min to 1700 ml/min the average dimensions of the droplet ranged from 67.93 up to 154.14 pixels. Based on the results obtained, it was concluded that the flow of nozzles has an impact on leaf coverage, droplet size and plant protection costs.

Keywords: *Nozzle testing, farming sprayer, quality of phytopharmaceuticals application, drip size, costs of plant protection.*

UVOD

Upotreba fitofarmaceutskih sredstava (FFS) u poljoprivrednoj proizvodnji zahtijeva visok stepen opreznosti. Konzumenti poljoprivrednih proizvoda, kao i poljoprivredni proizvođači trebaju biti svjesni svih opasnosti koje mogu biti prouzrokovane nestručnom primjenom FFS. Da bi zaštita bilja bila pravovremena, precizna i efikasna oprema sa kojom se vrši aplikacija FFS mora biti u ispravnom stanju. Pored toga koncentracija i vrijeme aplikacije su također bitni. Nepridržavanja navedenim načelima aplikacije FFS u poljoprivredi mogu dovesti do štetnih posljedica po zdravlje ljudi, zagađenja okoline i povećanja troškova poljoprivredne proizvodnje. Troškovi mogu biti direktni, koji se ogledaju u prekomjernom trošenju pesticida i indirektni koji se odražavaju na smanjene prinose i kvalitet proizvoda. U Bosni i Hercegovini su prisutne obje navedene pojave. Farmeri ne posjeduju znanja pravilne pripreme rastvora, doziranja i kontrole aplikacije FFS. Treba imati u vidu i zastarjelost poljoprivredne mehanizacije sa kojom se izvodi zaštita bilja u BiH. Fokus istraživanja ovoga rada usmjeren je na tehničku ispravnost dizni na uređajima za aplikaciju FFS. Obuhvaćeno je pet brendova dizni koje se najčešće koriste u BiH. Izvršeno je ispitivanje protoka dizni, kontrola kvaliteta aplikacije FFS i analizirani su troškov*i* zaštite bilja u ratarskoj proizvodnji. Dosadašnja istraživanja mnogih autora ukazuju da istrošene i nekvalitetne dizne mogu uticati na kvalitet aplikacije, a time i na troškove u poljoprivrednoj proizvodnji. Prema Đukiću i sar. (2001) dizne određuju veličinu kapljica, oblik i ugao izlaznog mlaza, količinu tečnosti i kvalitet pokrivanja tretirane površine.

Polazeći od navedenog stanja fokus rada usmjeren je na protok dizni, krupnoću kapljica i pokrivenost tretirane površine. Očekivani rezultati doprinijeti će sagledavanju uticaja tehničke ispravnosti dizni na kvalitet i racionalnije trošenje FFS u zaštiti bilja.

MATERIJAL I METOD RADA

Ispitivanje protoka dizni i kvaliteta aplikacije FFS je provedeno u ovlaštenoj "Laboratoriji za poljoprivrednu mehanizaciju" Poljoprivredno-prehrambenog fakulteta u Sarajevu. Ispitivanje protoka rađeno je na opremi za testiranje prskalica „*Schachtner tip control B20*" uz korištenje nošene traktorske prskalice „Agromehanika 330". Doza aplikacije FFS iznosila je 330 l/ha, visina prskanja bila je 50 cm i radni pritisak 3 bara. Ispitivanje je provedeno na novim diznama koje su najčešće prisutne na tržištu BiH. U eksperimentu je bilo pet brendova lepezastih dizni sa uglom prskanja 110°, protokom 1,6 l/min na pritisku 3 bara. Radilo se o izvedbama dizni od metala (CuZn) i plastike (PVC). Metalne dizne označene su oznakom (1) a plastične sa oznakom (2). U skladu sa postavljenim ciljevima, kvaliteta aplikacije FFS se mjerila digitalnom metodom analize slike vodeno-senzibilnih listića. Uslovi mjerenja i obrada digitalnog zapisa slike rađena je prema iskustvenim normama (Škaljić i Rakita, 2015.) koje su provjerene u odnosu na mikroskopski metod. U ispitivanju su korišteni vodeno-senzibilni listići formata 26 x 76 mm. Listići su pričvršćeni za bijeli papir formata A4 i postavljeni na ravnoj podlozi sa razmacima prilagođenim položaju dizni. Broj mjernih listića iznosio je 16, što je odgovaralo broju dizni na ispitivanoj prskalici. Tokom mjerenja traktor sa uključenom prskalicom kretao se preko mjernih listića brzinom od 6 km/h. Mjerni listići su prirodno sušeni a potom podvrgnuti postupku digitalnog snimanja pod kontroliranim uslovima visine, ugla i intenziteta vještačke svjetlosti. Korištena je prosječna rezolucija snimanja 3648 x 2736 piksela. Primijenjeni uslovi i način snimanja su bitni kod digitalne metode mjerenja (Rakita, 2008.), jer se u protivnom javljaju greške. U eksperimentu primijenjeni su sljedeći uslovi snimanja:

- Udaljenost kamere od objekta posmatranja bila je 50 cm.
- Izvor vještačke svjetlosti bio je reflektor sa bijelom svjetlošću koja daje sliku u njenim stvarnim bojama. Temperatura svjetlosti iznosila je 10.000 kelvina.
- Pohranjivanje digitalnog zapisa u JPEG formatu rezolucije 3648 x 2736 piksela.
- Obrada i analiza slike vršena softverom GIMP 2.8.

Za obradu i prezentiranje rezultata korišten je standardni *Microsoft Office Software*.

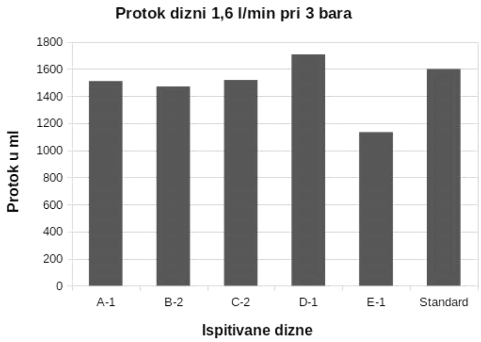
REZULTATI I DISKUSIJA

Istraživanjem je obuhvaćeno pet brendova dizni istih deklarisanih karakteristika, koje su nabavljene u redovnoj maloprodajnoj mreži poljoprivrednih apoteka u BiH. Imajući u vidu da na kvalitet zaštite bilja utiče tehnička ispravnost dizni i da na tržištu BiH postoji sumnja da se prodaju dizne bez deklaracije i provjere tehničke ispravnosti, izvršena su ispitivanja prema ranije objašnjenoj metodici rada. Statistički obrađeni rezultati ispitivanih dizni dati su u narednoj tabeli.

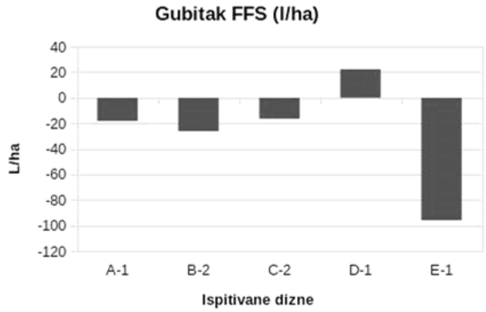
Tabela 1. Statistički pokazatelji protoka (ml) kod različitih dizni pri 3 bara.
Table 1. Statistical Flow Indicators (ml) for different nozzles at 3 bar.

Statistički pokazatelji	**Ispitivane dizne (1600 ml/min, pritisak 3 bara)**				
	Kosmos (1)	**AG (2)**	**Lechler (2)**	**Kovina (1)**	**Mlaz (1)**
N_0	15	15	15	15	15
Min. [ml]	1400	1400	1430	1660	1080
Max. [ml]	1590	1520	1580	1760	1180
Sred.vrij.[ml]	1511,33	1472,67	1519,33	1708	1134,67
Stand.devijac.	43,73	35,75	37,31	30,98	24,45
Varijansa	1912,38	1278,09	1392,38	960	598,09

Prema Sedlaru i sar. (2014) protok dizni sa oznakom 04 galona i pritisku od 3 bara iznosio je 1600 ml/min, uz relativno odstupanje od ±5%. Rezultati ispitivanja su pokazali da na tržištu kod novih dizni postoji odstupanja u odnosu na deklarisani protok. Najmanje prosječno odstupanje je zabilježeno kod dizni Lechler (2) (80,67 ml/min), koje je bilo u granici dozvoljenog. Prosječni protok dizni Mlaz (1) iznosio je 1134,67 ml/min što je mnogo manje od deklarisanog protoka (1600 ml/min), a dizne Kovina (1) su u prosjeku izbacivale 1708 ml/min što je 108 ml/min više u odnosu na deklarisanu normu. Dizne Kosmos (1) i AG (2) također nisu bile u skladu sa propisanom deklarisanom normom, što se može vidjeti na grafikonu 1 i 2.



Graf. 1. Prosječni protok dizni.
Graph. 1. Average nozzle flow.



Graf. 2. Prosječni gubitak FFS l/ha
Graph. 2. Average loss of FFS l/ha.

Protok FFS bio je manji kod brendova Kosmos-1 (-5,54%), AG-2 (-7,95%), Lechler-2 (-5,04%) i Mlaz-1 (-29,08%), dok su dizne brenda Kovina-1 izbacivale više tečnosti od deklarisane norme (+6,75%). Istraživanja Tadića i sar. (2012) navode da su imali odstupanje protoka kod dizni brendova *Mlaz* (+0,81 %), *Laznik* (-1,01%), *Andrić* (-64,44%) i AG (+3,70%) na pritisku 3 bara. Ako se ima u vidu da dozvoljena tolerancija protoka može biti ±5% onda su dizne Kosmos (1) i Lechler (2) bile blago iznad gornje granice dozvoljenog a preostale AG (2), Mlaz (1) i Kovina (1) su bile van dozvoljenih granica tolerancije.

U odnosu na podešenu dozu aplikacije FFS, koja je trebala biti 330 l/ha, odstupanja su bila manja kod dizni oznake Mlaz-1 (-95,96 l/ha), Kosmos-1 (-18,8 l/ha), AG-2 (-26,23 l/ha), i Lechler-2 (-16,63 l/ha), dok su dizne oznake Kovina-1 izbacivale više (+22,27 l/ha).

Analiza pokrivenosti mjernih listića bila je najbolja kod dizni Lechler-2, gdje je utvrđena prosječna pokrivenost od 22,46%, a najlošija pokrivenost bila je kod dizni Kosmos-1 od 9.78%. Dizna AG-2 davala je prosječnu pokrivenost od 15,02%, Mlaz-1 od 9,96% i dizna Kovina-1 od 18,48%. Dobiveni rezultati pokazuju da su dizne Kosmos-1 i Mlaz-1 u odnosu na diznu Lechler-2 imali duplo lošije prekrivanje. Slabije prekrivanje dovodi do smanjenog djelovanja FFS-a, što u konačnici izaziva lošu zaštitu i smanjene prinose. Istraživanja Bugarin i sar. (2006) su ustanovila da pri tretiranju korova na strništu sa dozom od 100 l/ha ostvaruju pokrivenost od svega 9,92%, prskanje dozom 200 l/ha ostvarilo je pokrivenost 14,12%.

Analiza veličine kapljica apliciranog fitofarmaceutskog sredstva je dala pokazatelje prezentirane u tabeli 2.

Tabela 2. Prosječne veličine kapljica apliciranog FFS u pikselima [px].
Table 2. Average size of drops of applied FFS in pixels (PX)

Statistički pokazatelji	Ispitivane dizne na radnom pritisku 3 bara				
	Kosmos (1)	**AG (2)**	**Lechler (2)**	**Kovina (1)**	**Mlaz (1)**
Br. mjerenja	90	90	90	90	90
Min.	9	3	7	6	8
Max.	521	395	1205	774	434
Sred. vrijednost	121,22	86,15	154,41	123,96	67,93
Stand. devijac.	107,57	86,15	154,41	123,96	67,68
Varijansa	11573,19	6931,05	36547,39	19656,98	4581,7

Prosječna veličina kapljica se kretala u rasponu od 67,93 px (Mlaz-1) do 154,41 px (Lechler-2). Veličina kapljica apliciranog FFS u direktnoj je vezi sa pokrivanjem tretirane površine i kvalitetom zaštite.

Tabela 3. Kvalitet aplikacije ispitivanih dizni.
Table 3. Quality of application of examined nozzles.

Kvaliteta zaštite	**Ispitivane dizne na pritisku 3 bara**				
	Kosmos (1)	**AG (2)**	**Lechler (2)**	**Kovina (1)**	**Mlaz (1)**
Prosječna pokrivenost tretirane površine u %	9,78	15,02	22,46	18,48	9,94
Prosječna nepokrivenost tretirane površine u %	90,22	84,98	77,54	81,52	90,06
Protok dizni u ml.	1520,00	1490,00	1530,00	1700,00	1140,00
Prosječna veličina kapljica u px.	121,22	86,15	154,14	123,00	67,93
Prosječan broj kapljica u isječku 100 x 100 [px]	19,66	24,33	16,33	19,33	27,00

Troškovi zaštite bilja mogu dodatno da opterećuju poljoprivredne proizvođače, pogotovo kad se radi sa neispravnim i nepodešenim mašinama za aplikaciju FFS. Prskanje sa neispravnim poljoprivrednim prskalicama, odnosno nepodešenim i neatestiranim utiče na kvalitet zaštite, zagađivanje okoliša i proizvodnju neispravne hrane. Potrošnja pesticida u poljoprivredi zavisi od vrste poljoprivredne proizvodnje i nekih drugih faktora, ali kod konvencionalnih tehnologija proizvodnje voća broj tretiranja se kreće od 5 do 15 pa i više. Ratarske i povrtlarske agrotehnike zahtijevaju niži broj tretiranja FFS i orijentaciono se kreće do 6 prskanja u vegetacionoj sezoni. Potrošnja pesticida u prosjeku se kreće po jednom tretiranju od 2-3 kg/ha.

Istraživanja Bugarin i sar. (2010) navode da se zaštita zasada jabuke u prosjeku prska 10–12 puta godišnje, a u nepovoljnim kišnim godinama i 15–16 puta. Istraživanja drugih autora a i rezultati ovog istraživanja ukazuju da protok dizni i broj prskanja imaju uticaj na količinu FFS a time i troškove zaštite bilja.

Ako se imaju u vidu cijene FFS u BiH, prosječne doze tretiranja i agrotehnički zahtjevi poljoprivrednih kultura, može se doći do pokazatelja troškova zaštite bilja. Prosječne doze zaštite, cijene FFS i troškovi tretiranja dati su u tabeli 4.

Tabela 4. Prosječne cijene pesticida i tretiranja na tržištu BiH.
Table 4. Average prices of pesticides and treatment on the BiH market.

Vrsta pesticida	Naziv preparata	Doza	Prosječna cijena preparata u KM/l	Cijena jednog tretiranja u KM	Prosječna cijena tretiranja u KM	Prosjek jednog tretiranja u KM
Herbicidi	Lumex	3,5-4 l/ha	35	140	64,37	54,12
	Adengo	0,5 l/ha	140	35		
	Combio	2-2,5 l/ha	25	62,5		
	Motiwel	0,5 l/ha	40	20		
Fungicidi	Anreplus	1,5 l/ha	32	48	79,2	
	Opus Team	1 l/ha	100	100		
	Caramba	1,5l/ha	80	120		
	Controllan	1 l/ha	120	120		
	Acord	1 l/ha	8	8		
Insekticidi	Rotor 1.25 E	0,5 l/ha	30	15	18,8	
	Fastac	0,1 l/ha	120	12		
	Calipso SC	0,1 l/ha	402	40,2		

Na temelju prikupljenih i obrađenih podataka troškovi prosječnog tretiranja pesticidima u Bosni i Hercegovini iznose 54,13 KM. Ako se ima u vidu da pojedine poljoprivredne proizvodnje imaju više tretmana zaštite u vegetacionoj sezoni, onda se može pretpostaviti da bi prosječni godišnji troškovi FFS u voćarstvu se kretali u rasponu od 270,6 KM/ha do 811,8 KM/ha dok u ratarskoj i povrtlarskoj proizvodnji bi to iznosilo 324,72 KM/ha. Iz navedenih pokazatelja se vidi da se radi o značajnim sredstvima i da je neophodno analizirati racionalnost trošenja FFS. Prethodno je istaknuto da su sprovedena istraživanja ustanovila odstupanja dizni od deklarisanih normi protoka. U narednoj tabeli je prikaz prosječnih gubitaka jednog tretiranja, prouzrokovanog odstupanjem od deklarisanog protoka dizne.

Tabela 5. Pokazatelj prosječnog gubitka izraženog u KM/ha po jednom tretiranju.
Table 5. Indicator of average loss expressed in KM / ha per treatment.

Dizne	**Kosmos (1)**	**AG (2)**	**Lechler (2)**	**Kovina (1)**	**Mlaz (1)**
Pesticidi	-3,00	-4,30	-2,73	3,65	-15,74
Herbicidi	-3,56	-5,11	-3,24	4,34	-18,71
Fungicidi	-4,39	-6,30	-3,99	5,34	-23,03
Insekticidi	-1,02	-1,47	-0,93	1,25	-5,37

Sve ispitivane dizne izuzev Kovina-1 su izbacivale manje tečnosti. Gubitak novca kod dizni Kovina-2 u prosjeku je iznosio 3,65 KM/ha. Ostali ispitivani brendovi dizni imali su smanjen kapacitet protoka, ispod zahtijevanih normi. Kod dizni Kosmos-1 i Mlaz-1 kapacitet protoka je bio toliko smanjen da bi aplikaciju FFS trebalo ponoviti, što bi prouzrokovalo dodatne troškove rada ljudi i potrošnje goriva. Ako se ima u vidu da BiH raspolaže sa 1.589.000 ha obradivih površina i da se na površini cca 10-15% sprovodi zaštita bilja, onda se radi o značajnim količinama nepotrebno bačenih FFS.

ZAKLJUČCI

Rezultati sprovedenih istraživanja ukazuju da protočni kapacitet dizni ima uticaja na kvalitet aplikacije fitofarmaceutskih sredstava, te da se indirektno odražava na troškove zaštite bilja. Na tržištu BiH u poljoprivrednim apotekama su prisutne dizne sumnjivog porijekla i kvalitete bez deklaracije čija protočna vrijednost ne odgovara deklarisanim normama. Predpostavlja se da porijeklo takvih dizni je krivotvoreno. Testirani brendovi dizni imali su odstupanja od deklarisanih normi. Manju količinu protoka od propisanoga imali su brendovi Kosmos-1 (-5,54 %), AG-2 (-7,95 %), Lechler-2 (-5,04%) i Mlaz-1 (-29,08%), dok je dizna brenda Kovina-1 izbacivala više tečnosti od deklarisane norme (+6,75 %). Kada je u pitanju kvalitet aplikacije FFS najbolja pokrivenost površine bila je kod dizni Lechler-2 (22,46%), a najlošija kod dizni Kosmos-1 (9,78%). Prosječnu pokrivenost imale su dizne AG-2 (15,02%), Mlaz-1 (9,96%) i dizna Kovina-1 (18,48%).

Treba naglasiti da lošije prekrivanje lisne površine dovodi do smanjenog djelovanja FFS, što u konačnici izaziva lošu zaštitu i smanjene prinose. Analiza troškova prouzrokovanih nepravilnim protokom na diznama pokazala je da četiri ispitivana brenda imaju manji protok od zahtijevanog i to u takvom obimu da bi trebalo ponoviti tretman zaštite, što bi imalo za posljedicu povećanje utroška radne snage, mašinskog rada i potrošnje goriva. Kod ispitivanog brenda Kovina-1 ostvarena je prekomjerna doza aplikacije, koja bi izbacivala 3,65 KM/ha FFS više od potrebnog.

Na temelju dobivenih rezultata preporučujemo pojačani inspekcijski nadzor prodaje dizni i intenziviranje periodično kalibriranje uređaje za zaštitu bilja

LITERATURA

Banaj, Đ., Duvnjak, V. (2000): Ispitivanje radnog potencijala ratarskih prskalica, Poljoprivreda, Vol. 5, Broj 2

Bugarin, R., Đukić, N., Sedlar, A. (2010): Uticaj tehnike za aplikaciju pesticida na zagađenje zemljišta, Savremena poljoprivredna tehnika, Vol. 36, No. 2, 107-116.

Bugarin, R., Đukić, N., Sedlar, A. (2010): Kvalitet tretiranja zemljišta herbicidima kompresorskim prskalicama, Savremena poljoprivredna tehnika, vol. 32, No. 2, 55-62.

Derksen, R. C., Breth, D. I. (1994): Orchard air-carrier sprayer application accuracy and spray coverage evaluations. App. Eng. Agri. 10(4). 463-470.

Đukić, N., Sedlar, A. (2001): Dobra prskalica, efikasna zaštita; Revija agronomska saznanja,

Đukić, N., Sedlar, A., Bugarin, R. (2008): Definisanje matematičkog modela habanja rasprskavača i njegov značaj za domaću proizvodnju, Savremena poljoprivredna tehnika, 34 (1-2), str. 63-71.

Đukić, N, Ponjičan, O, Sedlar, A. (2001): Novo u tehnici za zaštitu bilja; Savremena poljoprivredna tehnika, br. 27 (3-4), str. 122-130, 6, str. 48 – 50.

Jensen, P. K., Jorensen, L. N., Kirknel, E. (2001): Biological Efficacy of Herbicides and Fungicides Applied with Low – Drift and Twin – Fluid Nozzles, Crop Protection 20, 57 – 64.

Matthews, G. A. (1992): Pesticide application methods, 2nd edition, Blackwell, University of Oxford

Tadić, V., Banaj, Đ., Petrović, D., Horvat, D., Lukinac, J. (2012): Utjecaj tlaka i visine mlaznice na raspodjelu tekućine prskalice, Agronomski glasnik - Glasilo Hrvatskog agronomskoga društva, Vol.74 No.2-3 Listopad.

Sedlar, A., Bugarin, R., Đukić, N. (2014): Tehnika aplikacije pesticida, Izdavač Poljoprivredni fakultet Novi Sad, COBISS.SR-ID 213740556.

EFFECT (S) OF ATMOSPHERIC COMPONENTS SUCH AS UV RAYS AND OZONE (O_3) ON PLANTS: GENERAL APPROACH*

Hakan Ulukan[1]

Review paper

Summary

The population increasing rate continues, the world's population will be 9,0-9,5 billion by 2030-2050; and then we should be immediately increased of the plant output. But, it is not possible to do it avoiding the (negative) effect(s) of atmospheric components such as UV rays and Ozone (O_3) during the production period(s). UV rays have [UV-A (315-400 nm), UV-B (280-315 nm) and UV-C (100-280 nm)]. UV rays and Ozone (O_3) exists both in the troposphere and stratosphere. According to our knowledge, it is a major secondary air pollutant and is produced by a complex series of photochemical, biochemical and morphological reactions with the (NOx, which means various nitrous gases) and volatile organic compounds (VOCs). The earth has five atmospheric layers (towards space) as i) Troposphere (Bad O_3), ii) Stratosphere (O_3/Good O_3), iii) Mesosphere, iv) Thermosphere and v) Exosphere. From them, the second layer (Stratosphere; O_3 Layer/Good O_3) totally absorbs (UV-C) but releases (UV-B and UV-A); then, released rays enter to last layer (Troposphere; Bad O_3) which is closest to the ground. This layer melts and reacts with them, and the majority of UV-B absorbs, but not UV-A. End of cycle, nearly all of UV-A and some parts of UV-B rays easily arrive at the earth's surface. In fact, their effects (UV-A, UV-B and O_3) are not different from each other, even more, or less the similar; but UV-B's are much common in the plants. Generally, these kind effects can be classified as i) direct (morphological, phenological, mutation, etc. ii) indirect (biochemical, histological, aging, etc.) and iii) interactive. In this paper, information about the effects of atmospheric components such as UV rays and O_3 on the plants will be given in a general approach with visual materials.

Key words: *Atmosphere, UV rays, plants, Ozone (O_3)*

INTRODUCTION

One of the major global issues is to feed the rapidly increasing population. Under these conditions to be able to perform a sufficient production (esp. the plant production) is not possible due to some negative limited factors/stressors. According

---

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1]University of Ankara, Faculty of Agriculture, Department of Field Crops, 06110, Dışkapı, Ankara (Türkiye); e-mail: ulukan@ankara.edu.tr

to statistics, it (population increasing rate) is expected by 2050 will be 9,0-9,5 billion and this growth will be causing other important problems such as nutrition, shelter, feeding, etc. So, hence, there is a demand to increase food production needs. Particularly, all of them are rated at the level not only of field or vast areas, and regions but also at the crop layer. UV rays are divided into (3) main groups: i) UV-A (315-400 nm), ii) UV-B (280-315 nm), and iii) UV-C (100-280 nm). From them, nearly the entire UV-C ray is absorbed by the stratospheric Ozone (O_3) layer, but most of the UV-A does/did not induce/induce a biological threat (except sunburns), but UV-B is rather dangerous (Piri *et al.*, 2011; Suárez, 2014). Within them, the most dangerous one is UV-C ray. This one is completely absorbed by the stratospheric Ozone (O_3) layer while UV-A radiation is very little affected (UV-A does no harm to the plants; but the UV-B intensity is mostly affected by the thickness of the stratospheric Ozone layer and exactly this type of radiation is most harmful to the plants (Piri *et al.*, 2011). One of the major global issues is to feed the rapidly increasing population. Under these conditions to be able to perform a sufficient production (esp. the plant production) is not possible due to some negative limited factors/stressors. According to statistics, it (population increasing rate) is expected by 2050 will be 9,0-9,5 billion and this growth will be causing other important problems such as nutrition, shelter, feeding, etc. So, hence, there is a demand to increase food production needs. Particularly, all of them are rated at the level not only of field or vast areas, and regions but also at the crop layer. UV rays are divided into (3) main groups: i) UV-A (315-400 nm), ii) UV-B (280-315 nm), and iii) UV-C (100-280 nm). From them, nearly the entire UV-C ray is absorbed by the stratospheric Ozone (O_3) layer, but most of the UV-A does/did not induce/induce a biological threat (except sunburns), but UV-B is rather dangerous (Piri *et al.*, 2011; Suárez, 2014). Within them, the most dangerous one is UV-C ray. This one is completely absorbed by the stratospheric Ozone (O_3) layer while UV-A radiation is very little affected (UV-A does no harm to the plants; but the UV-B intensity is mostly affected by the thickness of the stratospheric Ozone layer and exactly this type of radiation is most harmful to the plants (Piri *et al.*, 2011).

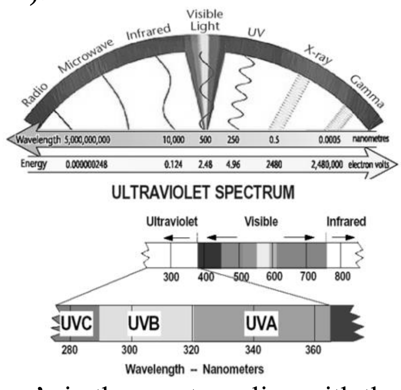
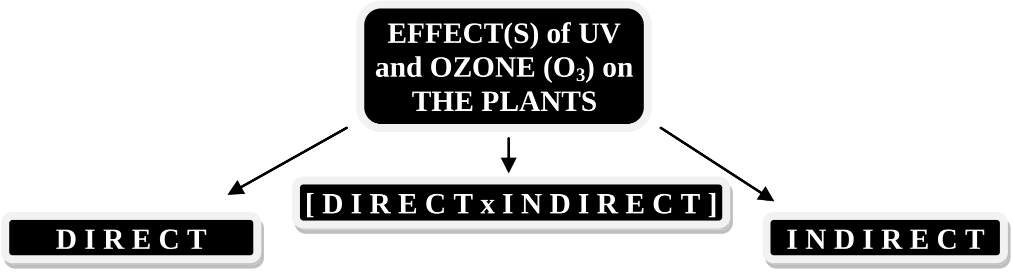


Fig. 1. UV rays place's in the spectrum line with the wavelengths (nm) (Anonymous, 2017a)

Some other component is Ozone (O_3). It is a natural gas that is found in two different layers in the atmosphere. In the layer around the Earth's surface, called the troposphere, bad Ozone pollutes the air and leads the fog or smog. The troposphere extends up to the stratosphere layer, where good Ozone protects like shield the of life by absorbing some of the UV rays. The planet has been wrapped in a blanket of air called the "atmosphere", which is made up of (5) different layers. Towards space they are; a) troposphere, b) stratosphere, c) mesosphere, d) thermosphere and e) exosphere. From the atmospheric components is Ozone (O_3), and it is located ≈ 19-30 km above the Earth as a layer of gas formed oxygen. Particularly, this layer is very important for whole living things because it (this layer) stops/blocks of the UV rays emitted from the sun. Among them, UV-B is much more common because its level directly depends on the O_3 layers (stratospheric and tropospheric), and its levels can be changed (due to Ozone depletion). UV-B effect(s)' can be classified as the morphological, physiological, and biochemical and DNA molecule damage and genotype of crops. Generally, effect(s) of the UV rays and Ozone (O_3) can be classified as i) direct (morphological, phenological, mutation, etc., ii) indirect (biochemical, histological, aging, senescence, etc.) and, iii) interactive (Kakani *et al*., 2003; Rastogi *et al*., 2010; Dzierzynska, 2012; Ali *et al*., 2016). In this review, information about the effects of atmospheric components of the UV rays and Ozone (O_3) on the plants will be examined as a general with their examples. Some other component is Ozone (O_3). It is a natural gas that is found in two different layers in the atmosphere. In the layer around the Earth's surface, called the troposphere, bad Ozone pollutes the air and leads the fog or smog. The troposphere extends up to the stratosphere layer, where good Ozone protects like shield the of life by absorbing some of the UV rays. The planet has been wrapped in a blanket of air called the "*atmosphere*", which is made up of (5) different layers. Towards space they are; a) troposphere, b) stratosphere, c) mesosphere, d) thermosphere and e) exosphere. From the atmospheric components is Ozone (O_3), and it is located ≈ 19-30 km above the Earth as a layer of gas formed oxygen. Particularly, this layer is very important for whole living things because it (this layer) stops/blocks of the UV rays emitted from the sun. Among them, UV-B is much more common because its level directly depends on the O_3 layers (stratospheric and tropospheric), and its levels can be changed (due to Ozone depletion). UV-Bs' effect(s) can be classified as the morphological, physiological, and biochemical and DNA molecule damage and genotype of crops. Generally, effect(s) of the UV rays and Ozone (O_3) can be classified as i) direct (morphological, phenological, mutation, etc., ii) indirect (biochemical, histological, aging, senescence, etc.) and, iii) interactive (Kakani *et al*., 2003; Rastogi *et al*., 2010; Dzierzynska, 2012; Ali *et al*., 2016). In this review, information about the effects of atmospheric components of the UV rays and Ozone (O_3) on the plants will be examined as a general with their examples.



DIRECT

i) Crop morphology, phenology, etc.
ii) Crop physiology, stomatal control, etc.
iii) Crop biochemistry, etc.
iv) Crop genotype
v) DNA, enzymes, etc.

INDIRECT

i) Survival, growth rate and reprod.
ii) Acting as antioxidant systems
iii) Stomatal control

Fig. 2. Schematic effect(s) of UV rays and Ozone (O_3) on the plants

UV RAYS' DIRECT AND INDIRECT EFFECT (S) ON THE PLANTS

All of the cultivated plants are classified as C_3, C_4, and CAM according to their fixing carbon number at the end of the photosynthetic activities' (Campillo *et al.*, 2012). From them, the C_3 plants are used for nutrition, feeding (such as wheat, barley, sunflower, etc.); But, the C_4 plants (such as corn, sugar cane, sorghum) are high yielded, and they need more water to be able to grow; and the CAM plants (such as papaya, cactus, sweet potatoes, etc.) are the desert plants. In nature, all the susceptible plants are affected. Generally, direct and indirect effects of the UV ray's (incl. Ozone gas (O_3) with their interactions is the (nearly) the same on the plants (meanwhile their interactions) are similar to each other and Ozone effect. On the other hand, depending on the technology level, which is used in this area, there are no much recent data on this issue. According to effect(s) on the plants, they can be classified as i) Direct, ii) Indirect and iii) Direct x Indirect. Nearly all the effect(s) or stimulations on the plants realizes mostly on their morphology, physiology, metabolism', and their genotypes, etc. But, this point is clear that all of these events are totally dependent on the plant's age, effected period length, dosage(s), affected part(s), development and growth stage(s), water capacity both in tissue(s) and in the soil, etc. But generally, the effects of the UV rays' can be mentioned as follows: Destruction of photosynthetic pigments, depression of photosynthetic activity, reduction in the chlorophyll (esp. Type b) content (10-70% - Dzierzynska, 2012), necrotic and chlorotic spots on the leaves, (leaf size) smelling, fading, discoloration and browning, hypocotyls reducing, leaves thickness increasing, decreasing the plant height and stem diameter, length of internodes, stomatal number, floral morphology, reduction in the plant height, leaf blade and area (esp. in the wheat - *Triticum* spp.), But increasing with the length of the column, increases in leaf number, leaf area and stem height in black cherry (*Prunus serotina*) (Ali *et al.*, 2016), cell division and pod set decreasing (Black *et al.*,

2007) and yield level, reducing (in any crop) are emerging. In the Tables 1-2, the effect(s) of the UV rays' and Ozone on the plants were summarized.

Tab. 1. Summary of the effects of the UV rays on plants (Teramura, 1983; Krupa and Kickert, 1989) and desirable outcome(s) in some plants which may be regulated by UV rays (Wargent and Jordan, 2013).

The plant characteristics and crop type	Enhanced UV and outcome(s)
Photosynthesis	Decreases in the many plants
Leaf conductance	No effect on the many plants
Water use efficiency (*WUE*)	Decreases in the most plants
Dry matter production and yield level	Decreases in the many plants
Leaf area	Decreases in the many plants
Specific leaf weight	Increases in the many plants
Crop maturity	No effect
Flowering	May inhibit or stimulate flowering in the some plants
Interspecific differences	Species may vary in degree of response
Interspecific differences	Response varies among cultivars
Drought stress	The plants become less sensitive to UV but not too tolerant to drought
Cereal grain crops	Breeding for manipulation of UV photomorphogen,Cultivar selection for optimization of photosynthetically efficient the plant architecture
Rice	Increased photosynthetic productivity
Tuber crops	Enhanced nutritional quality of harvestable components
Vegetables	The plant growth regulation, reduced losses from field transplantation
Vegetables, fruits	Improved taste, color and shelf-life, postharvest treatment for enhanced nutritional benefits/food safety
All crops	Deterrence of insect-pest feeding (e.g. Herbivore), Efficient management of beneficial insects (predators, parasitoids, pollinators), The plant pathogen control (e.g. Induced the plant resistance, direct mortality)

Basically, there are two types of damages of the UV rays: First, damage to DNA breaking the helix and mutations, and second, damage to the plant physiology and biochemistry (Ali *et al*., 2016). UV-A, like UV-B, has many inhibitory effects on the plants and the level of response varied within the species, exposure length of UV-

A and growth stage of the plants; chl (a) was more affected than cl (b) but, within them (UV rays); the most considered and important one is UV-B ray. The effect of UV-B can be classified at the level of morphological, physiological, biochemical and mutagenic (*sensu lato* DNA damage and changes in the genotype) as known. It caused some visual symptoms such as changes in leaf color and form, at the beginning of bronzing or brown spots, later chlorosis, necrosis, and drying happen. Demolition of the chlorophyll content varies up to 40% (Kakani *et al*., 2003) depending on the exposure period length, tissue situation, etc. In cotton (*Gossypium* spp.), it was reported that the leaf thickness is decreased under the effect of UV-B, ultrastructure of the leaf (palisade and mesophile cells) shape changes and expands, shorter and spongy, nearly all the photosynthetic pigment damage and reduce (in dicots 10-78%, in monocots 0-33%), epicuticular wax layer content increase (23-28%) in barley (*Hordeum* spp.), in bean (*Vicia* spp.), and in cotton (200%). Absorbing compounds increase from (10-300) % in agronomic crops, delaying in both seedling emergence and flowering of several crop plants, reducing mainstem and branch elongation rates, more compact and shorter of the plants, decreasing the plant height and reducing stem elongation (due to changes in the phytohormone levels, especially IAA in sunflower (*Helianthus anuus*), in leaf area (by a reduction in cell size), in cell number, division and expansion, modifying the reproductive or floral morphology of the crops, flowering increased, reducing anther number and pollen tube lengths (10-25%) in maize (*Zea* spp.), in rye (*Secale* spp.), and in tobacco (*Nicotiana* spp.) (Ali *et al*., 2016). The effect(s) of UV-B rays' and other relevant components such as O_3 are not uniform over the earth's surface their concentrations in the high latitudes (esp. for the Ozone (O_3) - such as Antarctic and Arctic regions) are 40-50% lower than the pre-1980 values; in the mid-latitudes (35-60°N and 35-60°S) are 3-6% lower than pre-1980 values; and at the equator show minimum changes (Kakani *et al*., 2003). Phenolic compounds soluble in the water, such as flavonoids, mainly anthocyanins, are anti-UV protective filter and due to the presence of phenols epiderma absorbs most of UV-B rays and the mesophyll cells of needles reaches 0% of UV-B, in grasses and leaves of dicotyledonous trees (3-12) % and herbaceous dicots (18-41) % (Mpoloka, 2008), the flowering is either trigerred or prevented, similarly the ripening process is not affected (Krupa and Jäger, 1996). Another vital component of the atmosphere is the Ozone (O_3) gas and it is present both in the troposphere and the stratosphere (Krupa, 1988). Its effect(s) is/are happens in two ways: the first one is filtering of the UV rays as Good (*Stratospheric Ozone*) and Bad (*Ground level Ozone or Tropospheric Ozone*) Ozone and second one is harmful DNA's mutagenic or skin carcinogenic effect(s)). On the other hand, first of all, it should be clarified that the Ozone layer (*Stratospheric Ozone*) which filters of the UV rays called "*Good Ozone*"; but the Ozone layer (ground level Ozone or tropospheric Ozone) which effects on the plants and causes the mutagens (e.g. DNA helix breaking, and so on) In humans called *Bad Ozone*". Ground level Ozone is a threat to food production as it has a negative impact on the yield and quality of important staple crops such as soybean (*Glycine* spp.), wheat and rice, maize and barley (Anonymous, 2017b).

Tab. 2. Common symptoms of O_3 induced foliar acute and chronic injury (Krupa *et al.*, 1998, 2000).

Acute injury	Chronic injury
Broad-leaved plants	
Bleaching (unifacial/upper surface or bifacial): Small unpigmented necrotic spots or more general upper surface bleaching. Palisade cells and, when the injury is more severe, upper epidermal cells collapse and become bleached.	Pigmentation (Bronzing): leaves turn red-brown to as phenolic pigment accumulation.
Flecking: small necrotic areas due to the death of palisade cells, metallic or brown, passing off to tan or may come alone as chlorophyll breaks down.	Chlorosis: may result from nongreen pigmentation gray or white.
Stippling: tiny punctate spots where a fe palisade cells are dead or injured may l white, black, red, or red-purple.	Premature senescence: early loss of leaves, flowers or fruit.
Bifacial necrosis: when the entire tissue through the leaf is killed, bifacial, dead areas develop ranging in in color from white to dark orange-red. While small veins are usually killed along with the other tissue, larger veins frequently survive. Conifers Banding: clear bands of chlorotic tissue on semi-mature needle tissue following O_3 episodes.	Flecking and mottling: flecking is the earliest of conifers. Mottling is generally associated with diffuse chlorotic areas interspersed with green tissue on first-year needles.
Tipburn: characterized by dying tips of young elongating needles. At first reddish brown in color, later turning brown, injury spreading from the tip.	Premature senescence: early loss of needles.

After this clarification, role(s) and importance(s) of the Ozone (O_3) layers can be explained. It is interesting that there is a similarity in terms effects of the Ozone (O_3) and the UV-B on the plants, and these factors generate Reactive Oxygene Species (ROS) formation and oxidative stress; when the Ozone (O_3), and the UV-B, and penetration into leaves results in reduced stomatal conductance and intensity of photosynthesis and under the influence of the O_3 and the UV-B and the WUE (*water use efficiency*) (Dzierzynska, 2012). The Ozone (O_3) in the troposphere and the stratosphere has different effects on life on the earth, depending on its location (Sadras and Calderini, 2015). More than 90% of the Ozone (O_3) exist is in the stratosphere layer and the remaining being in the troposphere layer. The Ozone (O_3) plays a beneficial role by absorbing solar ultraviolet radiation (UV-B) from reaching the earth's surface (Sadras and Calderini, 2015). Ozone causes damage by entering the leaf intercellular air spaces via stomatal conductance (less for C_4 plants)-wet cell wall surfaces, damaging radicals and signaling that accelerate senescence, causes chain reactions (Fuhrer *et al.*, 1989; Herstein *et al.*, 1995). Stratospheric Ozone plays a beneficial role by absorbing solar ultraviolet radiation (UV-B) from reaching the earth's surface (Asseng *et al.*, 2009). In the light of this information can ask that what is the effect(s) of the Ozone on plants? There are two possible answers to this question. One of them is i) the effect(s) of the good/stratospheric Ozone (O_3), and, another one is ii) the effect(s) of the bad/ground level/tropospheric Ozone. In the first one; namely, good/ground level/stratospheric Ozone layer's effect (indirect effects on the plants via UV rays), but the final one is directly on all existing creatures.

II) EFFECT(S) OF GOOD/STRATOSPHERIC OZONE

As mentioned above, the effect is indirectly on the plants since the main effect of the UV-A, UV-B and UV-C. At this Ozone, the effect or impact happens majority the refining ability of UV rays. On account of the stratospheric Ozone (O_3) layer, these effect, convert(s) into the weakened/attenuated radiation than enter to the atmosphere. After this penetration and refining stages, this time the effect(s) of the ground or bad or trophospheric Ozone (O_3) activates. As mentioned above, the effect is indirectly on the plants since the main effect of the UV-A, UV-B and UV-C. At this Ozone, the effect or impact happens majority the refining ability of UV rays. On account of the stratospheric Ozone (O_3) layer, this effect, convert(s) into the weakened/attenuated radiation than enter to the atmosphere. After this penetration and refining stages, this time the effect(s) of the ground or bad or tropospheric Ozone (O_3) activates.

I) EFFECT(S) OF BAD/GROUND LEVEL/TROPOSPHERIC OZONE

Ozone (O_3) enters the leaf internal spaces, then dissolves in the aqueous layer in the cells and easily reacts with the targets of the plasma membrane surface, amino acids, proteins, cell walls, etc. Current worldwide average tropospheric O_3 levels were about 50 nmol Mol-1 in the year 2000, already 25% above the threshold established for damage to the sensitive plants (Fuhrer *et al.*, 1997). The effect of the Ozone (O_3) can be summarized as follows: entry into leaves, and the immediate reactions that occur in the leaf apoplast, the protective mechanisms that may be activated (Fiscus *et al.*, 2005). Soybean, wheat, and rice are the most sensitive plants to the Ozone (O_3), but, maize and barley are moderately sensitive, and current crop cultivars tend to be more sensitive to the Ozone (O_3) than older varieties; the uptake of the Ozone (O_3)

by crops through the leaf pores is affected by weather, soil water availability and the plant development. Irrigation of crops during warm, sunny days can enhance the amount of the Ozone (O_3) taken up by the leaves (Anonymous, 2017c). The Ozone (O_3) causes negative effects along a number of the plant processes such as photosynthesis, water use efficiency, rate of senescence, dry matter production, flowering, pollen tube extension, output level, photosynthetic activity, decreasing stomatal opening, diminishing energy production in the photosystems, decreasing CO_2 assimilation (Krupa *et al*., 2000). As mentioned above, the Ozone (O_3) causes damage by entering the leaf intercellular spaces via stomata, where it reacts with compounds in the exposed wet cell-wall surfaces, and large doses of O_3 can induce programmed cell death (McGrath *et al*., 2015). One of the most common effects of the Ozone (O_3) is to promote of the leaf senescence; thus, in pastures or other types of grasslands' flowering processes may be increased (Fuhrer and Booker, 2003).

CONCLUSIONS

Here is the key question is to find out the plants (esp. cultivated ones) which one is/are tolerate/resist the UV rays and Ozone (O_3). Moreover, to tolerate/resistant trait also varies under field and greenhouse conditions among cultivar(s), in addition, changeability of this trait of the plants is another problem. Similarly, it is interesting that important factors such as global warming, climate change, UV rays, Ozone (O_3), salinity, water constraint(s), etc. caused the stresses on the resources that limiting the use of them (resources). But, whatever it is, we should find out, release and use the tolerant/resist cultivar(s) to the UV and Ozone (O_3) effect(s) in both production and breeding programs, even if we do not know their mechanism sufficiently. But, there are many uncertainties still exist on the effect of Ozone (O_3) on the plants, we do not know its mechanism, etc. The more data we have in this regard, the more successful we are and the better equipped we will be.

REFERENCES

Ali, H., Ghori, Z., Sheikh, S. and A. Gul. (2016): Effects of Gamma Radiation on Crop Production, K. R. Hakeem (ed.), Crop Production and Global Environmental Issues, pp. 27-78, DOI: 10.1007/978-3-319-23162-4_2.

Anonymous, (2017a): Plant breeding.blogspot.nl/2015/03, http://plant-breeding.blogspot.nl/2015/03/uv-b-radiation-stress-in-plants.html (Access date 09.11.2017).

Anonymous, (2017b): Global challenge network on Tropospheric Ozone, Agricultural and crop-effects of Ozone, https//www.ceh.ac.uk/sites/default/files/files/GNC_OzoneFactSheets_AgriCropEffects.pdf (Access date 11.06.2017).

Anonymous, (2017c): Center for Ecology & Hydrology, https://www.ceh.ac.uk/our-science/research-facility/solardomes-and-Ozone-field-release-system, solar domes and Ozone field release system (Access date 19.04.2017).

Asseng, S., Cao, W., Zhang, W. and Ludwig, F. (2009): Crop Physiology, Modeling and Climate Change: Impact and Adaptation Strategies, In: Sadras, V. O. and D. F. Calderini (Eds.), Crop Physiology: Applications for Genetic Improvement and Agronomy. Academic, San Diego; pp: 511-543.

Black, V. J., Black, C. R., Roberts, J. A. and Stewart, C. A., 2000. Impact of Ozone on the reproductive development of plants. New Phytology, (147), pp. 421-447.

Campillo, C., Fortes R. and Prieto, M. (2012): Solar Radiation Effect on Crop Production. Solar Radiation, Elisha B. Babatunde (Ed.), ISBN; 978-953-51-0384-4, InTech; pp. 167-195.

Dzierzynska, A. (2012): “Good” and “Bad” Ozone-evaluation on the basis of the plant reacts to Ozone, Chemical Didactic Ecology and Metrology, Vol 17 (1-2), 97-112.

Fiscus, L. E., Booker, L. F. and Burkey, O. K. (2005): Crop responses to Ozone: uptake, modes of action, carbon assimilation and partitioning, Plant, Cell and Environment, Vol 28; pp. 997-1011.

Fuhrer, J. and Booker, F. (2003): Ecological issues related to Ozone: agricultural issues, Environmental International, Vol 29; pp. 141-154.

Fuhrer, J., Lehnherr B. and Stadelmann, F. X. (1989): Luftverschmutzung und landwirtschafth-liche Kulturen in der Schwelz, Landwirtschaft Schweltz, Vol 2; pp. 669-673.

Fuhrer, J., Skarby, L. and Ashmore, M. (1997): Critical levels for Ozone effects on vegetation in Europe, Environmental Pollution, Vol 97; Pp. 91-106.

Herstein, U., Grünhage, L. and Jager, H-J. (1995): Assessment of past, present, and future impacts of Ozone and carbon dioxide on crop yields, Atmospheric Environment, Vol. 29 (16), 2031-2039.

Kakani, G. V., Reddy, R. K., Zhao, D. and Sailaja, K. (2003): Field crop responses to ultraviolet-B radiation; a review, Agricultural Forestry Meteorology, Vol 120; pp. 191-218.

Krupa, S. V. (1988): Atmospheric Ozone; Formation and effects on vegetation, Environmental Pollution, Vol 50 (1-2), 101-137.

Krupa, S. V. and Kickert, N. R. (1989): The Greenhouse Effect; Impacts of Ultraviolet-B (UV-B) Radiation, Carbon Dioxide (CO_2), and Ozone (O_3) on Vegetation, Environmental Pollution, Vol 61; pp. 263-393.

Krupa, S. V., McGrath, T. M., Andersen, P. C., Booker, L. F., Burkey, O. K., Chappelka, H. A., Chevone, I. B., Pell, J. E. and Zilinskas, A. B. (2000): Ambient Ozone and the Plant Health, Plant Disease, Vol 85 (1), 4-12.

McGrath, M. J., Betzelberger, M. A., Wang, S., Shook, E., Zhu, X-G., Long, S. P. and Ainsworth, E. A. (2015): An analysis of Ozone damage to historical maize and soybean yields in the United States, PNAS, Vol 112 (46), 4390-14395.

Mpoloka, S. M. (2008): Effects of prolonged UV-B exposure in plants, African J Biotechnology, Vol 7 (25), 4874-4883.

Piri, E., Babaeian, M., Tavassoli, A. and Esmaeilian, Y. (2011): Effects of UV irradiation on plants, African J Microbiology Research, Vol 5 (14), 1710-1716.

Rastogi, R. P., Richa, K. A., Tyagi, M. B. and Sinha, P. R. (2010): Molecular mechanisms of ultraviolet radiation induced DNA damage and repair, J. Nucleic Acids 2010; 32 Pp. doi: 10.4061/2010/592980

Sadras, O. V. and Calderini, F. D. (2015): Crop Physiology, Elsevier 32 James Town Road, NW1 7BY, United Kingdom., ISBN: 978-0-12-4171046, pp 547.

Suárez, M. O. L. (2014): Molecular responses of plants to solar UV-B and UV-A radiation, Academic dissertation, Faculty of Biological and Environmental Sciences Viikki Graduate School in Biosciences University of Helsinki, Finland, pp 49.

Teramura, A. H. (1983): Effects of ultraviolet-B radiation on the growth and yield of crop plants, Physiologia Plantarum, Vol 58; pp. 415-427.

Wargent, J. J. and Jordan, R. B. (2013): From Ozone depletion to agriculture; understanding the role of UV radiation in sustainable crop production, New Phytologist, Vol 197 (4), 1058-1076.

DISTRIBUTION OF COMMON RAGWEED (*Ambrosia artemisiifolia* L.) IN THE MUNICIPALITY OF KALESIJA*

RASPROSTRANJENOST AMBROZIJE (*Ambrosia artemisiifolia* L.) NA PODRUČJU OPĆINE KALESIJA

Mirha Đikić[1], Nihad Muhamedbegović[1], Drena Gadžo[1], Nedžad Karić[1], Fejzo Bašić[1], Nermina Sarajlić[2]

Preliminary communication

Summary

Common ragweed (*Ambrosia artemisiifolia* L.) is a hardy, herbaceous plant of the Asteraceae family. Due to its biological and ecological characteristics, this plant can grow in very unfavorable conditions in nonagricultural grounds and ruderal habitats, becoming a very widespread weed species in a number of agricultural crops. Apart from agricultural aspect, the common ragweed's pollen is one of the main causes of allergic diseases. The purpose of this work is to investigate the presence, number, height, phase of development and location of common ragweed in the area of Kalesija municipality and its arable and ruderal grounds. The prevalence of common ragweed was recorded via point quadrat method, which implies the identification of species, the number of individuals, height, mass and the phenophase. Floristic field records were taken in different habitats (stubbles, row crops, meadows and ruderal habitats) of Kalesija municipality. *Ambrosia artemisiifolia* was found in all four types of habitats, and was most common in ruderal sites, which is expected, due to the fact that this is the habitat it colonizes first. It was also found in row crops, mostly in potatoes and corn. The presence was recorded in a number of locations in stubbles as well, so further spread of common ragweed is to be expected in these habitats as well. The smallest number of common ragweed plants was found in meadows. Given the fact that common ragweed is already well-established in the area of Kalesija, it is necessary to undertake all available measures of suppression in order to prevent further spreading of this invasive plant species.

Key words: *common ragweed, invasive species, distribution, Kalesija*

---

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1]Faculty of Agriculture and Food Sciences, University of Sarajevo
[2]Faculty of Natural Sciences and Mathematics, University of Niš

Rezime

Ambrozija (*Ambrosia artemisiifolia* L.) je jednogodišnja zeljasta biljka iz familije Asteraceae, koja danas predstavlja jedan od najraširenijih i najopasnijih korova na poljoprivrednim površinama i ruderalnim staništima zbog svojih biološko-ekoloških karakteristika (brz rast i reprodukcija, visok disperzioni potencijal, dugovječnost sjemena u zemljištu). Polen ove vrste smatra se vodećim uzročnikom alergijskih reakcija. Cilj ovog rada je utvrditi prisustvo, brojnost, visinu, fazu rasta i razvoja i lokacije ambrozije na području općine Kalesija, na obradivim i ruderalnim površinama. Rasprostranjenost ambrozije evidentirana je metodom kvadrata, koji podrazumijeva identifikaciju vrste, broja jedinki, visine, mase i fenofaze. Floristički snimci na terenu su rađeni na različitim staništima (strnjišta, okopavine, livade i ruderalna staništa) na području općine Kalesija. Ambrozija kao invazivna korovska vrsta nađena je na sva četiri tipa staništa, a najbrojnija je bila na ruderalnim. Prisustvo ambrozije zabilježeno je i u okopavinama, najviše u krompiru i kukuruzu, ali i na strnjištima, tako da je za očekivati daljnje širenje i na ovim staništima. Najmanja zastupljenost zabilježena je na livadama. S obzirom na to da je ambrozija pronađena na sva četiri tipa staništa na području općine Kalesija neophodno je poduzeti sve raspoložive mjere suzbijanja ove korovske vrste u cilju sprječavanja njenog daljeg širenja.

Ključne riječi: *ambrozija, invazivne vrste, rasprostranjenost, Kalesija*

INTRODUCTION

Common ragweed (*Ambrosia artemisiifolia* L., Asteraceae) originates from North America and is one of the most aggressive invasive alien plants in Europe. According to Kovačević & Groman (1964), it was first introduced to Europe in 1863 and has been continuously expanding ever since. The rapid expansion of this species in Europe began after the World War I. It was first recorded on the territory of Bosnia and Herzegovina in 1939, in the outskirts of Derventa (locality of Osojci), on agricultural land (Maly, 1940). In recent years, common ragweed was found to be an aggressive weed in both ruderal habitats and arable land (Đikić *et al*., 2004; Đikić *et al*., 2005; Gadžo *et al*., 2008; Šarić *et al*., 2011; Ademović & Pezer, 2015; Suljić, 2015; Bašić *et al*., 2017), where it causes major damage to agricultural producers by reducing the quality and yield of crops. Kazinczi *et al*. (2008a) noted that the presence of 1, 2, 5 and 10 *Ambrosia artemisiifolia* plants per m^2 reduces the yield of sunflower by 4, 6, 21 and 33%, respectively. Its competitive skills have been confirmed by the research carried out by Maryuskina & Didyk (2002), who found that common ragweed can delay the development of rhizomatous grass species for at least one year. Trkulja *et al*. (2009) found that the *Ambrosia artemisiifolia* plants can grow up to 2 m tall in optimal environmental conditions. It reproduces exclusively by seed, which germinates in mid-spring, when the soil is warm enough. *Ambrosia* *artemisiifolia* blooms from July until the first frosts (Šarić, 1989; Điki ć *et al*., 2007), and a single plant can produce up to 62,000 seeds (Kazinczi *et al*., 2008b), which can remain viable for 40 years (Levente *et al*., 2003). On the territory of Bosnia and Herzegovina, *Ambrosia artemisiifolia* is most numerous in stubbles, where it forms dense communities. It is also common in ruderal habitats, riversides, green areas along roads and other disturbed sites (Bašić *et al*., 2017). So far, this weed species has not been investigated in the territory of Kalesija municipality. The aim of this work was to collect the preliminary data the presence of *Ambrosia artemisiifolia* plants in local communities of Kalesija municipality, in both arable and ruderal habitats.

MATERIALS AND METHODS

The survey of the presence and distribution of comon ragweed in the Minicipality of Kalesija was conducted between April and October 2016. The determination of the presence of common ragweed was conducted using the point square method (0.25 m^2) (Novak *et al*., 2009), which implies the identification of species, number of individuals, their height, mass and development phase. The coverage in each site was assessed by modified Braun-Blanquet (1965) method, with the following ratings:

- 1: up to 5% plants per square
- 2: 6 - 25% plants per square
- 3: 26 - 50% plants per square
- 4: more than 50% plants per square.

Tre survey was carreid out in fout habitat types: stubble, row crops, meadows and ruderal sites. A total of 30 phytocenological records were made in each habitat, except for ruderal sites, in which 55 records were made. All locations in which the common ragweed was found were recorded with GPS device. Number of individuals, the height of the tallest plant, mass of all individuals and recorded stage of development were recorded.

RESULTS AND DISCUSSION

During this research, the common ragweed was recorded in all parts of Kalesija municipality. Out of 145 visited sites, its presence was recorded in 109, most of which (51) were in ruderal habitat (Table 1).

Table 1. The presence of common ragweed in different habitats in Kalesija municipality

Habitat	Total number of records	Number and percentage of locations with common ragweed		Rating according to Braun-Blanquet							
	Number	**Number**	**%**	**1**	**%**	**2**	**%**	**3**	**%**	**4**	**%**
Stubble	30	16	14.68	2	12.50	5	31.25	6	37.50	3	18.75
Ruderal	55	51	46.79	2	3.92	9	17.64	18	35.29	22	43.13
Row crops	30	28	25.69	4	14.30	6	21.42	11	39.28	7	25.00
Meadow	30	14	12.84	3	21.42	6	42.85	4	28.57	1	7.14
Total	**145**	**109**	**100**	**11**	**10.09**	**26**	**23.85**	**39**	**35.77**	**33**	**30.27**

Common ragweed was found in 26 sites along the M4 main road, the Kalesija - Živinice regional road and several local roads. Given the facts that green areas along the main, regional and local roads in Bosnia and Herzegovina are not being mowed frequently enough (at least four times during vegetation period), and that the common ragweed seeds easily get transported by car wheels, it is clear why the expansion of this species is a serious problem. The highest density (328 plants m^{-2}) was recorded on ruderal site near the Old Mosque in Tojšići. The tallest plants (up to 2 m), and the highest measured mass (2.32 kg in Brezik) were also recorded in ruderal sites. Those robust plants produce a large amount of seeds, which is easily moved to agricultural and other sites by wind and water. In August and September, when the common ragweed was in full bloom, 10 cm tall seedlings were recorded in several locations, showing that this plant can be found in almost every development stage during the vegetation period. In urban areas common ragweed poses a threat to human health, due to highly allergenic pollen. In August 2007, Brkljač *et al*. (2008). measured a maximum concentration of 1021 pollen grains per m^3 of air in the area of Banja Luka, which is about 68 times higher than the concentration which Janjić *et al*. (2007) quote as the lower threshold in which allergic reactions start to occur. A large number of locations with common ragweed was also recorded in row crops (Table 1), mostly potatoes. The highest density (129 plants m^{-2}) in row crops was recorded in Rainci Donji. On several locations, the entire plot of land planted with potatoes was overgrown by this weed, probably due to the fact that weed control measures in potato crops are seldom undertaken in the area of Kalesija municipality. Herbicides are mostly used in corn production, resulting in it being less suspectible to weeds, including the common ragweed. The largest mass of plants (954 g m^{-2}) was recorded in potato plot in the very center of the city, and the tallest plants (up to 1 meter) were recorded in Kalesija Selo and Lipovice. Out of 30 visited stubble sites, common ragweed was recorded in 16 (Table 1). The largest mass (1.214 kg m^{-2}) and height (1.3 m) of plants in stubble was recorded in Miljanovci, along the main road M4, and the highest density (285 plants m^{-2}) in Rainci Donji. A large number of locations with common ragweed was also registered in Sprečko polje. In the area of Kalesija municipality, *Ambrosia artemisiifolia* was also found in meadows (in 14 out of 30 surveyed sites), meaning that it already began to compete with indigenous plants. At one of the sites visited, the coverage by common ragweed was over 50%, and the plants had a total mass of (0.546 kg m^{-2}). The highest density (172 plants m^{-2}) in meadow habitat was redorded in Lazarevići, and the highest plants (0.75 m) in Rainci Donji.

Figure 1 shows the overview of all locations with *Ambrosia artemisiifolia* in Kalesija municipality. It was found in all surveyed habitats, and was most numerous along the roads, but also in stubbles and row crops.

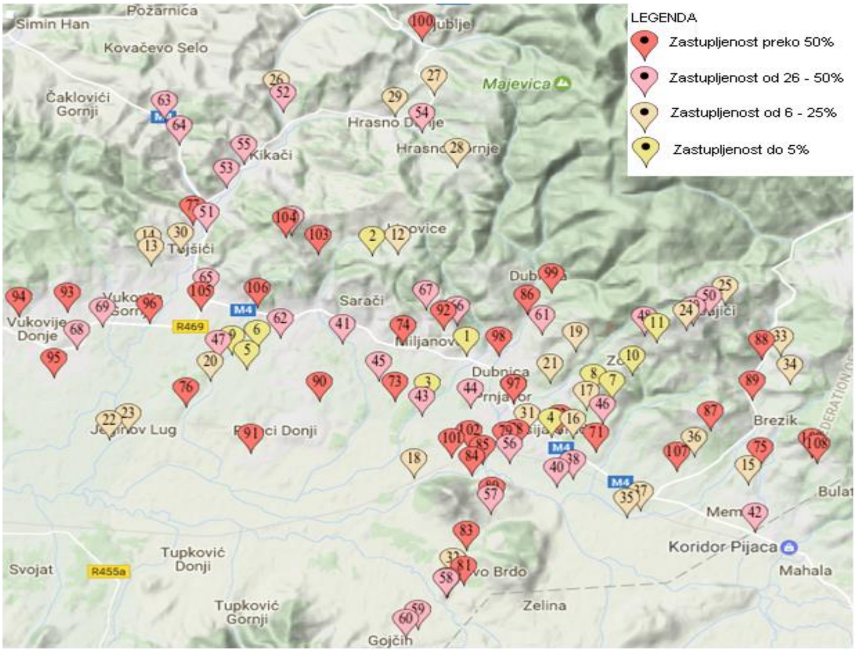


Figure 1. Distribution of *Ambrosia artemisiifolia* in the municipality of Kalesija

Common ragweed has been the subject of numerous researchers from Bosnia and Herzegovina. In 1986, Šarić *et al.* (1992) studied its distribution in Bosanska Krajina, and compared their results with the earlier data, concluding that *Ambrosia artemisiifolia* has rapidly spread in the fields of northern Bosnia, from the Sava River towards the south. It was registered in various crops, and the largest number of 200-300 plants per m^2 was recorded in stubble. Šilić & Abadžić (2000) found *Ambrosia artemisiifolia* in most localities in northern Bosnia where it was recorded earlier, but also in new locations in western Bosnia and in the Neretva valley. While investigating the weed flora in potato crops, Herceg (2003) determined the presence of common ragweed in two localities (Vitina near Ljubuški and Buna near Mostar), with an average 52 plants m^{-2} at the Buna site. According to Dakić (2005), common ragweed is a very common weed species in wheat, corn and other crops, but also in ruderal sites in the northwest Bosnia and Herzegovina. While investigating the distribution of major invasive weeds in the area of Sarajevo Canton, Suljić (2015), found *Ambrosia artemisiifolia* in all nine municipalities of the Canton, and it was most numerous in Hadžići municipality (250 plants m^{-2}). The analysis of the presence and distribution of *Ambrosia artemisiifolia* in the territory of Bosnia and Herzegovina shows its spread in arable land, ruderal and neglected sites, even in higher altitudes. It is agressively expanding in the northern part of Bosnia and Herzegovina, and is also present in the south, where it is less common, but with the tendency of further expansion (Bašić *et al.*, 2017). Weed control in row crops is mostly done by mechanical and chemical measures, but the extent of *Ambrosia artemisiifolia* spread indicates that these measures are not being implemented in the best possible way, and points to the need to take additional measures and procedures. The efficient control of *Ambrosia artemisiifolia* can only be achieved through integral approach, which involves the combined use of preventive, mechanical, agro-technical, physical and chemical control measures.

CONCLUSIONS

Common ragweed was found in all surveyed habitats in the area of Kalesija municipality, and was most numerous in ruderal sites. The presence of common ragweed along the roads enables its fast expansion, due to the fact that its seeds easily get transported by car wheels and that green areas along roads are not mowed as often as they should be. In Kalesija municipality, common ragweed is also common in row crops, mostly potato and corn, and in stubble. It was also recorded in meadows, where it competes with native vegetation. During this research, solitary plants were found in some locations, but in other dense communities were recorded, such as 328 plants m^{-2} in Tojšići. The efficient control of *Ambrosia artemisiifolia* can only be achieved through integral approach, which involves the combined use of preventive, mechanical, physical and chemical control measures. The eradication of this invasive weed should be imperative for everyone responsible for its presence and further reproduction, from farmers, companies responsible for maintenance of roads and urban greenery, and local population.

REFERENCES

Ademović, E., Pezer, L. (2015): Distribucija vrste *Ambrosia artemisiifolia* L. na širem području općine Mostar. Treći naučno-stručni skup sa međunarodnim učešćem "5. juni - Svjetski dan zaštite okoliša". Zbornik radova: 147-152, Bihać.

Bašić, F., Đikić, M., Gadžo, D. (2017): Appearance and spreading of common ragweed (*Ambrosia artemisiifolia* L.) in Bosnia and Herzegovina. Folia biologica et geologica (Ljubljana), 58(2): 5-13.

Braun-Blanquet, J. (1965): Plant sociology – The study of plant communities. Hafner Publisching Company, New York.

Brkljač, G., Trkulja, V., Ćurković, B., Rajčević, B. (2008): Monitoring koncentracije polena ambrozije na području grada Banje Luke tokom 2007. godine. Zbornik radova, Naučno-stručni skup savremene tehnologije za održivi razvoj gradova, 335-345, Banja Luka.

Dakić, P. (2005): Proučavanje korova u usjevu pšenice i kukuruza i mogućnosti njihovog suzbijanja primjenom herbicida. Magistarski rad. Poljoprivredni fakultet, Banja Luka.

Đikić, M., Gadžo, D., Muminović, Š. (2004): Značaj suzbijanja korovske vrste *Ambrosia artemisiifolia* na teritoriji Bosne i Hercegovine. Prvi simpozij Zaštita bilja sa pravcima daljeg razvoja, Sarajevo, BiH.

Đikić, M., Berberović, H., Gadžo, D., Huremović Z. (2005): Značaj suzbijanja ambrozije (*Ambrosia artemisiifolia*) kao korovske i alergogene vrste. Zbornik II Simpozija o zaštiti bilja u BiH, Teslić.

Đikić, M., Gadžo, D., Berberović, H., Petrović, D. (2007): Invazivne korovske vrste u Bosni i Hercegovini. Zbornik sažetaka IV Simpozij o zaštiti bilja u BiH, Teslić.

Gadžo, D., Gavrić, T., Đikić, M. (2008): Spreading of common ragweed in the Federation of Bosnia and Herzegovina. 2nd International Symposium „Intractable Weeds and Plants Invaders, Osijek, Hrvatska.

Herceg, N. (2003): Utjecaj kultivara i rokova sadnje na količinu i kakvoću priroda krumpira u Hercegovini. Doktorska disertacija. Agronomski fakultet, Mostar.

Janjić, V., Mataruga, D., Malidža, G., Radivojević, Lj., Mitrić, S., Stefanović, L., Stanković-Kalezić, R., Golić, D. (2007): Ograničenje širenja i suzbijanje ambrozije. U knjizi: "Ambrozija" (urednici: Janjić, V., Vrbničanin, S.), str. 103–118. Herbološko društvo Srbije, Beograd.

Kazinczi, G., Beres, I., Varga, P., Kovacs, I., Torma, M. (2008a): Competition between crops and *Ambrosia artemissiifolia* L. in aditive field experiment. Hungarian weed research and technology, 8(1): 41-47.

Kazinczi, G., Beres, I., Novak, R., Biro, K., Pathy, Z. (2008b): Common ragweed (*Ambrosia artemissiifolia*): a review with special regards to the results in Hungary. I. Taxonomy, origin and distribution, morphology, life cycle and reproduction strategy. Herbologia, 8(1): 55-93.

Kovačević, J., Groman, E. (1964): Korov limundžik (*Ambrosia artemisiifolia* L.) u Jugoslaviji. Zaštita bilja, 77, XV, 81-85, Beograd.

Levente, K., Laszlo, V., Gyula, B. (2003): A parlagfu (*Ambrosia artemisiifolia* L.) elleni biologiai vedekezes lehetosegi. Novenyvedelem 39, 319-331. 40.

Maly, K. (1940): Notizen zur Flora von Bosnien-Herzegovina. Glasnik Zemaljskog muzeja Bosne i Hercegovine, II, 1-2. Sarajevo.

Maryuskina, V. YA., Didyk, N. P. (2002): Experimental study of the interference between *Ambrosia artemisiifolia* L. and *Elytrigia repens* (L.) Nevski. Proceed. of the 12th EWRS Symposium, Wageningen, Netherlands, 288-289.

Novak, R., Dancza, I., Szentey, I., Karaman, J. (2009): Arable weeds of Hungary, Fifth national weed survey, Budapest.

Suljić, N. (2015): Rasprostranjenost važnijih invazivnih vrsta korova na području Kantona Sarajevo. Magistarski rad, Poljoprivredno-prehrambeni fakultet Sarajevo.

Šarić, T. (1989): Atlas korova. „Svjetlost“, Sarajevo.

Šarić, T., Elezović, Z., Muminović, Š. (1992): Ekspanzija divljeg sirka, ambrozije i smrdljive koprive u Bosni. Zbornik radova III kongres o korovima, Banja Koviljača, Srbija.

Šarić, T., Ostojić, Z., Stefanović L., Milanova, S., Kazinczi G., Tyšer, L. (2011): The changes of the composition of weed flora in Southeastern and Central Europe as affected by cropping practices. Herbologia, 12(1): 5-27.

Šilić, Č., Abadžić, S. (2000): Prilog poznavanju neofitske flore Bosne i Hercegovine. Herbologija, 1(1): 29-40.

Trkulja, V., Herceg, N., Ostojić, I., Škrbić, R., Petrović, D., Kovačević, Z. (2009): Ambrozija. Društvo za zaštitu bilja u Bosni i Hercegovini.

PLODNOST ZEMLJIŠTA REGIJE MODRIČKOG LUGA NAKON PROLJETNIH POPLAVA 2014. GODINE*

SOIL FERTILITY IN THE AREA OF MODRIČKI LUG AFTER 2014 SPRING FLOOD

Emina Sijahović[1]

Professional paper

Summary

In May 2014, heavy rain hit Bosnia and Herzegovina. After flooding, the samples of soil and sludge were taken from the plot in Modriča Lug, which were mainly sown with corn. Physical and chemical properties and heavy metals content of the sample were done. The following heavy metals were included: lead (Pb), cadmium (Cd), zinc (Zn), nickel (Ni), chrome (Cr), and copper (Cu). The content of heavy metals in the soil samples was determined by atomic absorption spectrophotometry.
Obtained values for the content of all examined heavy metals in the soils at the examined sites were significantly below the limit values stipulated by the law legislation in Bosnia and Herzegovina, indicating that the examined soils are not contaminated with heavy metals. It can be concluded that the floods did not significantly reduce the quality of the examined soil.

Key words: *Floods, soil analysis, heavy metals*

Rezime

Tokom mjeseca maja 2014. godine, velike kiše su pogodile Bosnu i Hercegovinu. Nakon poplava, sa parcela u Modričkom Lugu, koje su uglavnom bile zasijane kukuruzom uzeti su uzorci zemljišta i mulja, te im je urađena fizičko-hemijska analiza i sadržaj teških metala.
Istraživanjem su obuhvaćeni sljedeći teški metali: olovo (Pb), kadmij (Cd), cink (Zn), nikal (Ni), krom (Cr) i bakar (Cu). Sadržaj teških metala u ispitivanim uzorcima zemljišta određen je metodom atomske apsorpcione spektrofotometrije. Utvrđene vrijednosti za sadržaj svih ispitivanih teških metala u zemljištima na ispitivanim lokalitetima su bile znatno ispod graničnih vrijednosti propisanih zakonskom legislativom u Bosni i Hercegovini, iz čega se može zaključiti da ispitivana zemljišta nisu onečišćena teškim metalima.

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27th-29th September 2017, Sarajevo, Bosnia and Herzegovina

[1] Gulf real estate doo

Ključne riječi: *poplava, analiza zemljišta, teški metali*

INTRODUCTION

In mid-May 2014 major floods occurred after the rainfall that had exceeded the record for the last 120 years, since first measurements were made (www.capital.ba, 2014). The rivers Bosna, Drina, Sana, Sava, Vrbas and others poured out of their basins. The May floods were of catastrophic proportions in BiH. When it comes to damages in agriculture, which is still one of the most important branches of business in BiH, 70,000 hectares of cultivable land and crops were affected by floods in different levels of development (www.capital.ba, 2014).
Land is one of the key constraining factors of a plant production due to the simultaneous work of two processes, increasing the need for food, on the one hand, and reducing the area of agricultural land on the other (Kljajić *et al*., 2012). Production capacity of land is being reduced by processes of continuous decline and damage of agricultural areas. Preservation of agricultural land, as the primary resource for food production, is the basis for the progress and stability of the development of every society. In order to prevent the degradation of biological, chemical and physical properties of the soil, primarily caused by anthropogenic factors, it was necessary to take appropriate measures to maintain soil fertility for a longer period and at the same time prevent or at least reduce contamination of the soil with undesirable harmful substances.
Among the harmful substances in the soil, heavy metals are particularly distinguished by their negative effects, because they can have a very negative effect on human health (Ali *et al*., 2004; He *et al.*, 2005). In this case, especially distinguished, because of their negative effects, are cadmium (Cd), chromium (Cr) and lead (Pb), which, if found in food or drink, can cause a number of problems in the human organism functioning (Pan *et al*., 2010). Unlike the aforesaid, some heavy metals such as copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) are essential for the normal functioning of a human organism, but only if they are taken in small quantities, and their presence is, to some extent, desirable in soil and food (Linder *et al*., 1996).
This paper presents the results of soil analysis of samples taken from the site Modrički Lug after the floods, which was sown with corn.

MATERIAL AND METHODS

After the floods, the field in the Modriča Lug area was visited, where the water remained at the level of 2 m for four days, and samples were taken for chemical analysis of soil fertility and analysis of heavy metals content in order to determine the degree of soil contamination. This paper presents sample analyzes from 3 localities belonging to Modrički Lug, Vukosavlje Municipality, Cadastral Zone Modriča: Garevac, Prudić and Brezik. Three average samples were taken from each site,

obtained from 15 individual samples at a depth of 0 - 30 cm. As a part of the chemical analysis, the following parameters were tested: soil acidity (pH reaction), humus content, phosphorous and potassium-free resin and as the most important parameter; the content of the total forms of heavy metals; Cd, Cr, Pb, Zn, Cu and Ni in the soil. The acidity of the soil was determined by the pH meter (ISO 10390, 1994), the content of the humus by the dihalogen method (ISO 14235, 1994), the content of the free-form potassium and phosphorus AL method (Egner *et al.*, 1960), and the content of the total forms of heavy metals by the flame atomic absorption spectrophotometry method on Shimadzu AA-7000 (ISO 11047, 1998). The extraction of heavy metals from the soil was made by using 'aqua regia' (mixtures of nitrate and hydrochloric acid in a ratio of 1:3) and according to the instructions contained in the ISO 11466 method (ISO 11466, 1995).
The basic legal legislation used for this part of the research was: 'Ordinance on Determination of Permissible Quantities of Harmful and Dangerous Substances in the Land for Federation of BiH' (2009).

RESULTS

The results of the analysis of the basic soil fertility parameters on the surveyed parcels are presented in Table 1.

Table 1. The results of chemical analyses of soil from the examined area

Sample	**Location**	**pH in H_2O**	**pH in KCl**	**Humus, %**	**K (mg/100g)**	**P (mg/100g)**
1A	Garevac	6.3	5.4	2.2	33.9	0.101
2A	Garevac	7.8	6.5	1.9	44.6	0.053
3A	Garevac	8.0	7.4	2.4	18.1	0.048
1B	Brezik	8.0	7.3	1.2	19.0	0.028
2B	Brezik	7.6	6.9	2.4	38.7	0.081
3B	Brezik	7.4	6.3	2.3	60.1	0.038
1C	Prudic	7.3	6.4	2.8	19.6	0.026
2C	Prudic	7.7	7.1	1.8	27.0	0.135
3C	Prudic	7.4	6.5	2.6	24.0	0.071

Based on the results of the soil fertility analysis and the measured pH value, it can be concluded that this is neutral to slightly alkaline soil and from this point of view it is favorable for corn cultivation. The content of easily accessible phosphorus is low and potassium is optimal. The content of humus is very low. It is recommended to introduce phosphorus and potassium as well as organic fertilizers prior to basic soil treatment. It is recommended to fertilize with mineral NPK fertilizer formulation 7:20:30 in an amount of 500 kg ha^{-1}. The required amount of nitrogen can be achieved by nitrogenous fertilizer urea in an amount of 200 kg ha^{-1}. Fertilizers should not come into direct contact with seeds. The second fertilization treatment, during the vegetation, should be done with 100 - 150 kg ha^{-1} of KAN or 50 kg ha^{-1} urea.

Table 2. The content of heavy metals in soil samples at the examined area

Sample	**Sample**	**Ni**	**Zn**	**Pb**	**Cr**	**Cu**	**Cd**
1A	Garevac	**71.54**	71.73	37.19	59.88	17.19	0.9
2A	Garevac	**191.8**	118.51	33.81	11.37	33.70	0
3A	Garevac	**226.7**	109.34	25.92	91.52	38.51	-0.01
1B	Brezik	20.8	70.58	24.23	12.43	12.29	-0.02
2B	Brezik	**201.5**	106.99	54.66	55.36	41.78	-0.02
3B	Brezik	33.15	78.54	34.94	23.73	32.84	-0.01
1C	Prudic	**143.2**	75.63	27.05	**101.12**	27.34	-0.01
2C	Prudic	**94.8**	86.21	34.94	34.18	24.41	-0.01
3C	Prudic	23.8	74.08	23.11	10.17	12.55	-0.02
REFERENCE (permissible) values of total forms of heavy metals in soil (mg kg soil) ˉ1		**50**	**200**	**100**	**100**	**80**	**1.5**

Based on the results of the soil samples analysis taken from agricultural areas that were affected by the floods in May 2014 and the obtained results, the following can be noted:

The content of heavy metals: zinc (Zn), lead (Pb), chromium (Cr), copper (Cu) and cadmium (Cd) in all samples is within the limits of the permissible values, except: The content of nickel (Ni) in most of the samples analyzed was above the permissible limits, especially in the samples: 1A; 2A; 3A; 2B; 1C; 2C. Šaćiragić (2005), states that accessible forms of nickel in the soil range up to 2.5% of total nickel concentrations, and considers that affordable quantities of nickel are not toxic to vegetation, as these soils contain enough calcium and a high pH that reduces toxicity of larger quantities of nickel. Boyle *et al*. (1988), indicate that this heavy metal is not a limiting factor for the production of a health-worthy food. In addition, this is an essential element in plant nutrition, so its presence to certain amounts in plants is indispensable. The content of chromium (Cr) was slightly above the allowable value in Prudić, sample 1C.

It can be stated that there is no serious risk of soil contamination of analyzed soil samples contamination; therefore it may be recommended the use of the mentioned land from which sampling for agricultural production was taken.

CONCLUSION

Based on the analysis of all results in this research, the following conclusions were drawn:
- The content of all tested heavy metals was significantly below the limited values prescribed by the legal legislation in BiH, except nickel content.
- Due to the determined heavy metal content, the land at the investigated sites can be classified as a land with very low levels of heavy metal contamination.
- Examined localities meet all the criteria needed for successful corn cultivation observed from the aspect of soil contamination with heavy metals, which is also the basic conclusion of this study.
- After the floods and the analysis, the users of the parcels were given recommendations for further use of land for plant production and its protection.

LITERATURE

Ali, N. A., Ater, M., Sunahara, G. I., Robidoux, P. Y. (2004): Phytotoxicity and bioaccumulation of cooper and chromium using barley in spiked artificial and natural forest soils. Ecotoxicology and environmental Safety, 57: 363-374.

Boyle, R. W., Robinson, H. A. (1988): Metal Ions in Biological Systems, Vol. 2.

Egner, H., Riehm, H., Domingo, W. R. (1960): Untersuchungen uber die chemische Bodenanalyseals Grundlage fur die Beurteilung de Nahrstoffzustandes der Boden. II. K. LantbrHogsk 26:199-215.

He, Z. L., Yang, X. E., Stoffellab, P. J. (2005): Trace elements in agroecosystems and impacts on the environment. Journal of Trace Elements in Medicine and Biology, 19: 125-140.

International Standard Organisation (1994): Soil quality - Determination of pH.ISO 10390.1994(E).

International Standard Organisation (1995): Soil quality - Extraction of trace elements soluble in aqua regia. ISO 11466.

International Standard Organisation (1998): Soil quality - Determination of organic carbon by sulfochromic oxidation. ISO 14235.1998(E).

International Standard Organisation (1998): Soil Quality - Determination of cadmium, chromium, cobalt, copper, lead, manganese, nickel and zinc. Flame and Electrothermal AAS. ISO 11047

Kljajić, N., Arsić, S., Mijajlović, N. (2012): Land as an ecological factor of agricultural production, Transition, Vol.14 (29), 38 – 47.

Linder, M. C., Hazegh Azam, M. (1996): Copper biochemistry and molecular biology. Am. J. Clin. Nutr., 63: 797-811.

Official Gazette of FBiH (2009): 'Ordinance on Determination of Permissible Quantities of Harmful and Dangerous Substances in the Land for F BiH', no. 72/09.

Pan, J., Plant, J. A., Voulvoulis, N., Oates, C. J., Ihlenfeld, C. (2010): Cadmium levels in Europe: implications for human health. Environmental Geochemistry and Health, 32: 1-12.

Šaćiragić, B. (2005): Agrohemija, Sarajevo.

http://www.capital.ba/ukupne-ekonomske-posljedice-poplava-u-bih-398-milijardi-km/)

ORGANSKA PROIZVODNJA MESA PERADI*

ORGANIC POULTRY MEAT PRODUCTION

Alma Rustempašić[1], Admir Dokso[1], Ervin Zečević[1]

Professional paper

Rezime

Organska proizvodnja mesa peradi zahtijeva posebne uslove uzgoja, koji su propisani standardima za organsku proizvodnju, te predstavlja brojne izazove za uzgajivače. Jedan od važnih preduslova za uspješnu organsku proizvodnju je upotreba pasmina koja je prilagođena sistemu organske proizvodnje. Aktivnost, vitalnost, otpornost na bolesti, omjer konverzije hrane dovode do upotrebe sporih i srednje rastućih pasmina u usporedbi s hibridima koji se koriste u intenzivnim proizvodnim sistemima. Pri izboru pasmina u organskoj peradarskoj proizvodnji se daje prednost autohtonim pasminama i sojevima. Uzimajući u obzir da hibridi brzo rastu, te da mogu imati zdravstvene probleme koji negativno utječu na dobrobit životinja.
U radu će se opisati osnovna načela organske proizvodnje peradi i pravilan izbor pasmina za proizvodnju mesa.

Ključne riječi: *organska proizvodnja, perad, meso*

Summary

Increased consumer interest in organic poultry products is evident in recent years. In response to consumer demands, in the EU has increased the number of organic poultry farms. Organic production of broilers meat is arranged in accordance with the principles laid down for this type of production and poses many challenges for farmers. One of the important preconditions for successful production is the use of breeds that are adapts to the organic production system. Activity, vitality, resistance to some disease, feed conversion ratio, lead to preference of slow and medium growing hybrids compared to hybrids used in intensive production systems. In the selection of breeds in organic poultry production, preference is given to indigenous breeds and strains. Taking into account that the fast growing hybrids in free range conditions may face some health problems which negatively affect the animal welfare, the correct choice of the breed is one of the key issues. This paper will describe the basic

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27th-29th September 2017, Sarajevo, Bosnia and Herzegovina
[1] Poljoprivredno-prehrambeni fakultet Sarajevo

principles of organic poultry production and the correct choice of breeds for meat production.

Key words: *poultry, organic production, meat*

UVOD

Razvoj proizvodnje u konvencionalnom peradarstvu išao je u pravcu povećanja kapaciteta farmi, izgradnje zatvorenih objekata, uvođenja mehanizovanih uređaja, kaveznog i baterijskog uzgoja, korištenja krmnih smjesa za ishranu i sa raznim aditivima i hormonima za ubrzavanje rasta. Namirnice proizvedene na intenzivan način mogu da sadrže značajne količine antibiotika što može negativno utjecati na ljudsko zdravlje. U javnosti su prisutna upozorenja ekologa o visokom stepenu zagađenosti životne sredine, a među zagađivačima se ističe farmska stočarska proizvodnja. Ovo ukazuje na povratak prirodnom, odnosno sistemu uzgoja peradi, koji zadovoljava biološke i etološke zahtjeve, koji su u skladu sa zakonskim propisima. Osnovni principi organske proizvodnje se odnose na dobrobit životinja, očuvanje zdravstvenog stanja, zaštiti životne sredine, očuvanje biodiverziteta, ograničenu upotrebu lijekova, te proizvodnju kvalitetnih animalnih proizvoda (Kijlstra i Eijck, 2006). Organska proizvodnja pilećeg mesa može se sažeti kao proizvod slobodnog sistema uzgoja koji u potpunosti izbjegava upotrebu konvencionalno uzgojenih životinja, uključujući genetski modificirane organizme, nusproizvode životinjskog porijekla i sintetičke aditive, uz ishranu organski uzgojenim krmivima.

SISTEMI PROIZVODNJE MESA PERADI

U proizvodnji pilećeg mesa koriste se industrijski sistemi: podni i baterijski, te neindustrijski (ekstenzivno u objektu, slobodan ispust, tradicionalno slobodan ispust, neograničen slobodan ispust i organska proizvodnja (Gančić i sar., 2000).
Pri organskoj proizvodnji mesa peradi pilići se drže u zatvorenom objektu do 28 dana uzrasta na podu s prostirkom kao što je slama, pilota, pijesak ili treset (maksimalno 10 pilića po m^2) u stacioniranim objektima, odnosno 16 pilića po m^2 u pokretnim kokošinjcima. Ukupna upotrebna površina objekata za perad za proizvodnju mesa ne prelazi 1600 m^2. Ni jedan peradarski objekt neće sadržavati više od 4800 kokoši. Potrebno je da imaju izlazne otvore dovoljno velike i to najmanje 4 m na 100 m^2 objekta. Poslije toga pa do kraja tova (81 dan) pilićima treba obezbijediti travnati ispust (minimalno 4m^2/grlu) pašnjak, ograđen kako bi se spriječio upad grabljivica tokom dana. Kao osnovna hrana mogu poslužiti žitarice, kukuruz, ječam, pšenica, dovoljne količine čiste i svježe vode. Životinje će biti hranjene organskom hranom koja zadovoljava nutritivne potrebe životinje u svim stadijima razvoja. Dozvoljena je primjena imunoloških veterinarskih lijekova. U zimskom periodu u kokošinjac je potrebno postaviti lampe koje osim što daju svjetlost, zagrijavaju prostor. Perad ne smije biti držana u kavezima (EK, 889/2008).

Vrijeme koje je neophodno da prođe da bi farma sa konvencijalnog prešla na organski način proizvodnje zove se prelazni period ili period konverzije. Da bi proizvod peradarske farme bio označen kao „organski“, neophodno je da životinje od kojih proizvodi potiču budu uzgajane po principima organske stočarske proizvodnje najmanje 10 sedmica (za perad za proizvodnju mesa), dovedenu prije dostizanja starosti od tri dana. Ako na tržištu nema dovoljan broj životinja organskog porijekla, a jato se formira po prvi put, mogu se uključiti i jedinke iz konvencionalne proizvodnje. Normativi organske stočarske proizvodnje propisuju da ukupna količina proizvedenog stajnjaka ne smije preći 170 kilograma azota godišnje po hektaru korištene poljoprivredne površine. Da bi se ispunio pomenuti normativ maksimalan broj brojlera po hektaru ne bi smio preći 580 jedinki (EK, 889/2008). Jedna od značajnijih prednosti stacioniranog kokošinjca u odnosu na pokretni je ta što su kokoške uvijek na istom mjestu u kokošinjcu ili na ispustu uz kokošinjac i nema potrebe za svakodnevnim hodanjem da bi se kokoške premještale, hranile i napajale. Glavna prednost prenosnih kokošinjaca je da se kokoške mogu prenijeti na dio pašnjaka sa svježom hranom, a pri tome se čestim premještanjem smanjuje rizik od kontakta pilića sa zemljištem u kome uvijek mogu da se nađu patogeni mikroorganizmi i paraziti koji izazivaju infekcije i bolesti. Nedostatak je što se pri premještanju kokošinjca mora premještati hrana, voda, prostirka i dr. (Čengić-Džomba i sar., 2014).

IZBOR ŽIVOTINJA ZA ORGANSKU PROIZVODNJU

Za organsku proizvodnju peradi je bitan izbor životinja, te se koriste vitalne vrste i pasmine koje su adaptirane na lokalne uslove uzgoja, izvore hrane i otporne na bolesti. Jedan od zdravstvenih problema koji se javljaju pri korištenju brzorastućih pasmina kokoši je pojava šepavosti (Fanatico i sar., 2008; Rack i sar., 2009; Tuyttens i sar., 2008). Smrtnost u organskoj proizvodnji brzorastućih pasmina je veća i može se zaključiti da spororastuće pasmine kokoši su pogodnije za organsku proizvodnju (Fanatico i sar, 2008; Castellini i sar., 2002). Pasmine sporijeg prirasta, koje se preporučuju u organskom peradarstvu imaju nižu stopu smrtnosti, otpornije su i aktivnije. Postoje razlike u ponašanju između brzorastućih i spororastućih pasmina. Spororastuće pasmine su aktivnije i često provode više vremena vani (Almeida i sar., 2012). U organskoj proizvodnji pilećeg mesa pilići se trebaju uzgajati 81 dan, odnosno dnevni prirast ne bi trebao biti veći od 50 g. U ispustima perad koristi i dopunska krmiva, kao što su zelena hrana, sjemenke, insekti, crvi i sl. Na ovaj način se obezbjeđuje dio proteina, vitamina i mineralnih materija, što poboljšava kvalitet mesa. Neke od spororastućih pasmina koje se preporučuju su Robusta Maculata, Kabir, Rowan Range, Sasso pasmine. Pri izboru pasmina u organskoj peradarskoj proizvodnji se daje prednost autohtonim pasminama i sojevima. U Bosni i Hercegovini postoji autohtona pasmina kokoši. To je domaća bosanskohercegovačka kokoš ili kako se još naziva pogrmuša ili živičarka. To je sitna kokoš najčešće crne ili jarebičaste boje, može biti i raznobojna u svim kombinacijama do potpuno bijele. Kasnozrela je pasmina, otporna i izdržljiva, kombinovanih svojstava mesa i jaja (Adilović, Andrijanić, 2005).

ZAKLJUČAK

Jedan od važnih preduvjeta za uspješnu proizvodnju je upotreba pasmina koja je prilagođena sistemu organske proizvodnje. Otpornost, aktivnost, vitalnost, te prirast ne veći od 50 g po danu, nas upućuju prema pasminama sporijeg rasta, te autohtonim pasminama koje su prilagođene na klimatske uslove i ishranu. Standardi organske proizvodnje zahtijevaju dosta ulaganja u proizvodnju od smještaja, travnatih ispusta, kvalitetne ishrane a imaju za cilj očuvanje zdravstvenog stanja i dobrobit životinja. Stoga se nadamo da će u budućnosti biti farmi koje će proizvoditi meso peradi na organski način što predstavlja poseban izazov za farmere.

LITERATURA

Adilović, S., Andrijanić, M., (2005): Bosansko-hercegovačke autohtone pasmine domaćih životinja, Veterinarski fakultet. Sarajevo.

Almeida, G. F. D., Hinrichsen, L. K., Horsted, K., Thamsborg, S. M. & Hermansen, J. E. (2012): Feed intake and activity level of two broiler genotypes foraging different types of vegetation in the finishing period. Poultry Science 9, 2105-2113.

Castellini, C., Bosco, A. D., Mugnai, C., Bernardini, M. (2002): Performance and behaviour of chickens with different growing rate reared according to the organic system. Italian Journal of Animal Science 1, 291-300.

Čengić-Džomba, S., Drkenda, P., Đikić, M., Gadžo, D., Latinović, M., Mirecki, N., Mirecki, S. (2014): Organska poljoprivreda, Biotehnički fakultet u Podgorici, Univerzitet u Crnoj Gori.

Kijlstra, A., Eijck, I. A. M. (2006): Animal health in organic livestock production systems: a review. Journal of Life Sience, 54,1,77-94.

Fanatico, A. C., Billai, P. B., Hester, P. Y., Falcone, C., Mench, J. A., Owens, C. M., Emmert, J. L. (2008): Performance, Livability, and Carcass Yield of Slow- and Fast-Growing Chicken Genotypes Fed Low-Nutrient of Standard Diets and Raised Indoors or with Outdoor Access. Poultry Science 87, 1012-1021.

Gančić, M., Krečov, M., Mašić, B. (2000): Tov pilića, Poljoprivredna biblioteka, Beograd.

Rack, A. L., Lilly, K. G. S., Beaman, K. R., Gehring, C. K. Moritz, J. S. (2009): The effect of genotype, choice feeding, and season on organically reared broilers fed diets devoid of synthetic methionine. Journal of Applied Poultry Research, 18, 54-65.

Službeni list Evropske Unije EK br. 889/2008.

Tuyttens, F., Heyndrickx, M., De Boeck, M., Moreels, A., Van Nuffel, A., Van Poucke, E., Van Coillie, E., Van Dongen, S., Lens, L. (2008): Broiler chicken health, welfare and fluctuating asymmetry in organic versus conventional production systems. Livestock Science 113, 123-132.

SMART FOOD PACKAGING*
"PAMETNA" AMBALAŽA HRANE

Enisa Omanović-Mikličanin[1]

Professional paper

Summary

The food packaging is used to protect food from the influence of external environmental conditions like heat, light, presence or absence of moisture, pressure, microorganisms, etc. It provides the consumer with greater ease of use and time-saving convenience. The key safety goal for traditional packaging materials which come into contact with food is to be as inert as possible. Technology innovations move the packaging market from conventional packaging to interactive, aware, and intelligent systems. These smart packaging concepts are based on the useful interaction between the packaging environment and the food ingredients to provide active protection to the food. Smart packaging utilizes chemical sensors or biosensors to monitor the food quality and safety from the farm to the consumers. It may result in a variety of sensor designs that are suitable for monitoring of food quality and safety (freshness, pathogens, leakage, carbon dioxide, oxygen, pH, time or temperature). It is needed as online quality control and food safety in terms of consumers, authorities and food producers. The addition of nanoparticles to food packaging systems may improve characteristics like barrier properties of packaging to different gasses, antimicrobial properties, biodegradability, or may achieve sensors properties that can inform on the food quality, etc. Currently, food packaging materials are the largest category of nanotechnological applications in the food sector.
Smart packaging has great potential in the development of new sensing systems integrated into the food packaging, which are beyond the existing conventional technologies. Therefore, this paper is aimed to make a survey of ongoing scientific research, technology development, and its influence on smart packaging design and implementation.

Key words: *Food, smart packaging, nanoparticles, nanomaterials, technology.*

Rezime

Ambalaža hrane se koristi kako bi zaštitila hranu od uticaja okoliša kao što je toplota, svjetlost, prisustvo ili odsustvo vlage, pritisak, mikroorganizmi, itd. Ona olakšava

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Faculty of Agriculture and Food Sciences, University of Sarajevo, Bosnia and Herzegovina

korištenje hrane. Osnovna odlika tradicionalnih materijala koji se koriste za pakovanje je da budu što inertniji kada su u kontaktu sa hranom. Tehnološke inovacije su pomjerile tržište materijala koji se koriste za ambalažu hrane u smjeru interaktivnih i intelegentnih sistema. Osnovni koncept ove „pametne" ambalaže hrane je interakcija između sredine u kojoj se vrši pakovanje i sastojaka hrane kako bi se obezbijedila aktivna zaštita hrane. „Pametna" ambalaža koristi hemijske senzore i biosenzore u praćenju kvaliteta i sigurnosti hrane u lancu od farme do potrošača. Postoji veliki broj senzora koji se mogu koristiti za monitoring kvaliteta i sugurnosti hrane (određivanje svježine, patogena, karbon dioksida, kisika, pH, vremena i temperature). Ovi senzori su neophodni za „online" kontrolu kvaliteta i sigurnosti hrane od strane potrošača, vlasti i proizvođača hrane. Dodatak nanočestica u sisteme za pakovanje hrane može spriječiti prodiranje gasova u ambalažu, poboljšati antimikrobne osobine pakovanja, biodegradibilnost, ili može dati informacije o kvaliteti hrane. Trenutno, nanotehnologija ima najznačajniju primjenu u oblasti pakovanja hrane.
„Pametna" ambalaža ima veliki potencijal u razvoju novih sistema u senzorima i biosenzorima koji se koriste za pakovanja hrane i time ima niz prednosti u odnosu na tradicionalne materijale koji se koriste za ambalažu hrane.
Ovaj rad predstavlja pregled savremene literature, istraživanja i tehnološkog razvoja i njihovog uticaja na dizajn i primjenu pakovanja hrane.

INTRODUCTION

Packaging provides containment and protect quality of food products during transportation, storage, and end use. It protect food from external and internal unfavorable conditions such as water vaporu, microorganism, gases, dust, mechanical shock and vibration[1,2].
As society is becoming increasingly complex, users (food producers, food precessors, logistic operators, retailers and consumers) are continuosly demanding innovative and creative food packaging to guarantee food safety, quality and traceability. This requires application of new technologies in food packaging processes. To make food packaging innovations commercialy viable and successfully adopted by the target group, they must meet the ever increasing regulatory requirements and have a final beneficial outcome that outweighs the possible extra expenses of adding the new technology. In addition, food packaging innovations should aim at decreasing the harmful environmental impact by taking in account a broad range of sustainability issues (waste prevention, efficient use of resources, reuse, recycle etc.)[3].
Nanoscience and nanotechnology have huge potential to bring benefits to many areas of research and application, such as computer electronics, communication, energy production, medicine and the food industry. The current stage of nanotechnology applications in the global food sector is exclusively modest and most products and applications are still at research and development stage. Still, there is a wide range of potential applications where nanotechnology could offer innovative solutions to the food and associated sectors (Fig. 1)[4].

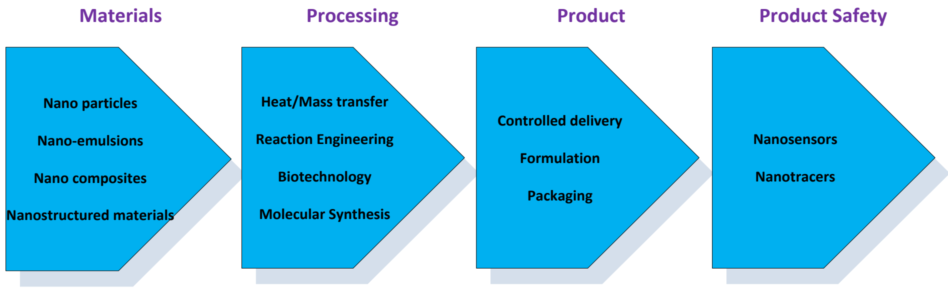


Figure 1. The main areas of nanotechnology application in food related sector[4]

Applications of nanotechnology in the area of food packaging and food safety resulted in **"smart packaging"** appearance in the market. Smart packaging can be divided into two categories: **active packaging** that provides functionality such as moisture and oxygen control, and **intelligent packaging** that can communicate product changes and other information. This paper surveys the smart packaging state of the art, advantages over traditional food packaging, risks and concerns.

SMART PACKAGING OR TRADITIONAL FOOD PACKAGING?

Traditional food packaging consumes mainly petroleum based plastics. The main risks and concerns of traditional food packaging production and applications are related to the non-sustainable production, lack of recyclability, insufficient mechanical and barrier properties. Those materials are not biodegradable too. Main challenges faced by many food producers are weak barrier properties to water vapour and gases and achieving and adequate shelf-life for the food products [2].

Nanotechnology plays a major role in filling the gaps of traditional packaging materials through smart packaging. Application of nanoparticles in food packaging positively affects the shelf-life, quality, safety, and security of food which ultimately benefit both the producers and consumers. "Smart packaging" presents a total packaging concept that combines the benefits arising from active and intelligent technology which include nanoparticle application in one or more production phases. The main risk associated with nanoparticle application is their migration into to the food that can potentially result in adverse health effects [5].

ACTIVE PACKAGING

Active packaging materials are materials that serve to extend the shelf-life or to maintain or improve the condition of packaged food. They are designed to incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food [6]. Different active substances can be incorporated into the packaging material, such as O_2 and ethylene scavengers, moisture regulators, CO_2 scavengers and emitters, antioxidant and antimicrobial controlled-release packages [7].

1 Extension of food shelf-life using antimicrobial nanoparticles and nanocomposites

Metal nanoparticles (silver, gold, zinc or metal oxides) have been used in various packaging applications. Due to their small size and surface reactivity, they can endow packaging materials with antimicrobial activity to prevent the proliferation of spoilage and pathogenic microogranisms [8]. Researchers have tried to produce antimicrobial silver nanocomposites [9].

2 Oxygen and ethylene scavenger

Oxygen in food package can lead to a reduction to product shelf-life caused by various degradation processes. Thus, oxygen within the package should be eliminated or reduced to a level acceptable for a given product. Other compounds released by fresh fruits and vegetables during storage can be undesirable too. In particular, ethylene vapor in climacteric fruits can accelerate the post-harvest ripening, quickly shortened the shelf-life. Various active packaging concepts have been applied for oxygen and ethylene scavenging [2].

3 Other types of active packaging materials

Biopolymers present the future of food packaging. According to the European Bioplastics, biopolymers are materials made from renewable resources which are biodegradable and compostable, thus they can act as fertilizers and soil conditioners. The performance expected from bioplastic materials applied in food packaging is protecting food from the environment and maintaining food quality [10].
Recently, many research studies have focused on developing **active packaging containing natural antioxidants**. Natural extracts of rosemary, oregano and green tea with antimicrobial and antioxidant properties have been used in active packaging systems to increase the stability of different meat products and to extend their shelf-life [11, 12].

INTELLIGENT PACKAGING

Intelligent packaging materials are materials that monitor the condition of packaged food or the environment surrounding the food [6]. Intelligent packaging, in a broader meaning, is defined as science and technology that use communication function of packaging system's to facilitate decision making by monitoring changes in the internal and external environments and communicating the conditions of the packaged food products [13]. Intelligent packaging does not directly act to extend the shelf-life of foods like active packaging. It aims to convey information to the stakeholders of the food supply chains related to the food quality. Intelligent packaging can be used to check effectiveness of active packaging systems. It can be said that active packaging is the component that take some action, while intelligent packaging is the component that senses and shares the information [1].

Intelligent packaging systems can be realized by three main technologies: indicators, data carriers and sensors. **Indicators** aim to provide more convenience and/or to inform consumers about the food quality. They can be reasonable included within three categories: time-temperature indicators, freshness indicators, and gas indicators. **Data carriers** are specifically intended for storage, distribution, and traceability purposes. They are known as automatic identification devices. The most important data carrier devices are barcode labels and RFID tags, which belong to the main category of convenience-enhancing intelligent systems [1]. **Sensors** are considered to be the most promising and innovative technology for future intelligent packaging systems. A sensor is a device or system with control and processing electronics, an interconnection network, and software. It is used to detect, locate, or quantify energy or matter, by giving a signal for the detection or measurement of a physical or chemical property to which the device responds. Different kinds of sensors intended for food applications have been developed [14, 15].

MARKET AND LEGISLATIVE CONSIDERATION

The European and US regulatory concepts about materials in contact with food (food-contact materials) differ in details and in fundamental approach. The European approach is based on the fact that all materials should be explicitly cleared and publicized in regulations. All materials should pass toxicological evaluation. The US approach gives considerable credibility to the idea that “the dose makes the poison” so that toxicological justification of all food-contact materials is not necessary [16].

Regulatory requirements for new active and intelligent packaging technologies are very similar in EU and US. Materials used in food-contact applications are subject to premarket regulatory clearance by the US Food and Drug Administration. The regulation of active and intelligent packaging in Europe is still evolving. Initially, all European food-contact legislation originated in and was applied in individual member states. Today, member states elected to harmonize legislation in order to create a single market. The EU regulation 1935/2004 offered for the first time the opportunity for “smart packaging” to be used in Europe by allowing the application of materials with agents that could migrate into foods. This Regulation contains general provisions on the safety of active and intelligent packaging [16].

One of the main issues that prevent higher application of smart packaging in food packaging is consumer’s acceptance of non-edible items separate from the package. Consumers are worried that smart packages might mislead them regarding the product’s quality [1].

CONCLUSIONS

The interest in innovative packaging systems to achieve higher food quality and safety, consumer convenience, and management along the food supply chain has boosted the development of „smart packaging“ Smart packaging can be divided into two categories: active packaging that provides functionality such as moisture and oxygen control, and intelligent packaging that can communicate product changes and other information. "Smart packaging" presents a total packaging concept that combines the benefits arising from active and intelligent technology which include nanoparticle application in one or more production phases.
Active packaging materials are materials that serve to extend the shelf-life or to maintain or improve the condition of packaged food.
Intelligent packaging materials are materials that monitor the condition of packaged food or the environment surrounding the food.
Regulatory requirements for new active and intelligent packaging technologies are very similar in EU and US. The EU regulation 1935/2004 offered for the first time the opportunity for "smart packaging" to be used in Europe by allowing the application of materials with agents that could migrate into foods.
Although the potential advantages arising from this new technology have been recognized, there is still an existing gap in market applications. For this reason, future research needs to consider some important aspects in order to make „smart packaging“ systems commercially viable and, ultimately, into everyday packaging commodities.

REFERENCES

1. Ghaani, M., Cozzolino, C. A., Castelli, G., Farris, C. (2016): An overview of the intelligent packaging technologies in the food sector. Trends in Food Science & Technology 51; 1-11.
2. Mihindukulasuriya, S. D. F., Lim, L. T. (2014): Nanotechnology development in food packaging: A review. Trends in Food Science & Technology, doi: 10.1016/j.tifs.2014.09.009
3. Vanderroost, M., Ragaert, P., Devlieghere F., De Meulenaer, B. (2014): Intelligent food packaging: The next generation. Trends in Food Science & Technology 39; 47-62
4. Omanović-Mikličanin, E., Maksimović, M., Vinković Vrček, I., Mulaomerović, Dz., Dorić, A. (2016): Application of Nanotechnology in Food Packaging, Proceeding book of 5th Workshop: Specific methods for food safety and quality, Satellite event of the 13th International Conference on Fundamental and Applied Aspects of Physical Chemistry - PHYSICAL CHEMISTRY; 119-125
5. Echegoyen, Y., Nerin, C. (2013): Nanoparticle release from nano-silver antimicrobial food containers. Food and Chemical Toxicology 62; 16-22

6. European Commission (2004) Commission Regulation (EC) No 1935/2004 of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directive 80/590/EEC. Official Journal of the European Union, OJ L 338, 4-17
7. Vermeiren, L., Devlieghere, F., van Beest, M., de Kruijf N., Debevere. J. (1999) Developments in the active packaging of foods. Trends in Food Science & Technology 10; 77-86
8. Bikiaris, D. N., Triantafylldis, K. S. (2013): HDPE/Cu-nanofiber nanocomposites with enhanced antibacterial and oxygen barrier properties appropriate for food packaging applications. Materials Letters 93; 1-4
9. Rhim, J. W., Wang, L. F., Lee, Y., Hong, S. I. (2013): Bionanocomposites for food packaging applications. Progress in Polymer Science; 1629-1652
10. Siracusa, V., Rocculi, P., Romani, S., Dalla Rosa, M. (2008): Biodegradable polymers for food packaging: a review. Trends in Food Sceince & Technology 19; 634-643
11. Barbosa-Pereira, L., Angulo, I., Lagaron, J. M., Paseiro-Losada, P., Cruz, J. M. (2014): Development of new active packaging films containing bioactive nanocomposites. Innovative Food Science and Emerging Technologies, doi: 10.1016/j.ifset.2014.06.002
12. Gomez-Estaca, J., Lopez-de-Dicastillo, C., Hernandez-Munoz, P., Catala, R., Gavara, R. (2014): Advances in antioxidant active food packaging.Trends in Food Science & Technology 35; 42-51
13. Yam, K. L. (2012): Intelligent packaging to enhance food safety and quality in Yam, K. L., Lee, D. S.:Emerging food packaging technologies: Principles and practice (p. 139). Philadelphia: Woohhead Publishing Limited
14. Gao, F., Zheng, D., Tanaka, H., Zhan, F., Yuan, X. (2015): An electrochemical sensors for gallic acid based on Fe2O3/electro-reduced grapheme oxide composite: estimation for the antioxidant capacity index of wines". Materials Science and Engineering 57; 279-287
15. Goulart, L. A, Cruz de Moraes, F., Mascaro, L. H. (2016): Influence of the different carbon nanotubes on the development of electrochemical sensors for bisphenol A. Materials Science and Engineering: C. 58; 768-773
16. Restuccia, D., Spizzirri, U. G., Parisis, O. I., Cirillo, G., Curcio, M., Iemme, M. Puoci, F., Vinci, G., Picci, N. (2010): New EU regulation aspects and global market of active and intelligent packaging for food industry applications. Food Control. 21; 1425-1435

AN ANALYSIS OF ECONOMIC ASPECTS OF GREENHOUSE TOMATO GROWING IN TURKEY*

Sait Engindeniz[1], Dilek Yucel Engindeniz[2], Murat Yercan[1], Filiz Kinikli[1]

Professional paper

Summary

Vegetable production is dominant in greenhouse production in Turkey, accounting for 95.3% of total greenhouse production, and that is followed by flower (3.5%) and fruit production (1.2%). Tomatoes were grown in 57% of total gerenhouse area in 2015. 77% of greenhouse tomatoes was produced in plastic greenhouses. Greenhouse tomato production increased 64% in 2006-2015 period. The purpose of this study is to analyse economic aspects of greenhouse tomato growing in Turkey. For this aim, data of 2006-2015 period were investigated. Data were collected from Turkish Statistical Institute, The Turkish Ministry of Food, Agriculture, and Livestock, The Turkish Ministry of Environment and Urbanisation, and Mediterranean Exporter Associations General Secretariat. Besides, results of previous studies were also used. Growers should gather all the economic data about the greenhouse tomato production, and market conditions before building a greenhouse.

Key words: *greenhouse, greenhouse vegetables, tomato, cost analysis, economic analysis.*

INTRODUCTION

Greenhouse tomato growing plays an important role both from the perspective of the farm, as well as from that of the entrepreneur in Turkey. Greenhouse production provides fresh produce for consumers on a year-round basis. It provides income for small-scale farmers. It requires intensive labour and input use in the production process and as a consequence it encourages labour efficiency. Further, it is also very important in creating a demand for subsectors that provide inputs for greenhouse production such as seed, fertilisers, pesticides, glass, etc. (Yilmaz *et al*., 2005).
Greenhouse tomato production is very popular in many areas of the World. With more than 7,500 varieties of tomatoes, trying to choose the right type for greenhouse production can be a daunting task. However, certain varieties of tomatoes are bred to grow specifically in greenhouse conditions and produce fruit for longer periods of

*Rad prezentiran na 28. Međunarodnoj naučno-stručnoj konferenciji poljoprivrede i prehrambene industrije / Paper presented at the 28th International Scientific-Expert Conference of Agriculture and Food Industry, 27–29 September, 2017, Sarajevo, Bosnia and Herzegovina
[1] Ege University, Faculty of Agriculture, Department of Agricultural Economics, 35100 Bornova-Izmir, Turkey.
[2] Dokuz Eylul University Izmir Vocational High School Agricultural Business Management Program, 35160 Buca-Izmir, Turkey.

time than other cultivars. When choosing which variety to grow, consider the size of the fruit the plant produces.

In recent years, many studies have been made on economic analysis of greenhouse tomato growing in Turkey (Engindeniz and Tuzel, 2002; Engindeniz, 2003; Rad and Yarsi, 2005; Karaman and Yilmaz, 2006; Engindeniz *et al.*, 2009). But, the studies on the economic aspects of the greenhouse tomato growing should be carried out continuously.

The purpose of this study is to analyse economic aspects of greenhouse tomato growing in Turkey. For this aim, data of 2006-2015 period were investigated. Data were collected from Turkish Statistical Institute, The Turkish Ministry of Food, Agriculture, and Livestock, The Turkish Ministry of Environment and Urbanisation, and Mediterranean Exporter Associations General Secretariat. Further, results of previous studies were also used.

DEVELOPMENT OF GREENHOUSE TOMATO PRODUCTION IN TURKEY

Turkey is one of the major greenhouse production countries in the World. Turkey lies between 36 and 42° North and 26 and 45° East. The main advantage of Turkey in terms of wide spreading of greenhouse growing is mild winter climate which compared to the countries located at higher latitudes is characterized by relatively high radiation and mild temperatures during winter (Tuzel and Leonardi, 2009).

Eighty percent of total greenhouses in Turkey are covered with plastic and the rest with glass. The total protected cultivation area of Turkey was about 66,362 ha including 38,941 ha by glass and plastic greenhouses in 2015. Vegetable production is dominant in greenhouse production in Turkey, accounting for 95.3% of total greenhouse production, and that is followed by flower (3.5%) and fruit production (1.2%) (Tuzel *et al.*, 2015). Among the vegetables, tomato is the most prominent while cucumber, pepper, eggplant, squash, watermelon, melon and other vegetables are grown over rest of the area.

Tomatoes are grown in autumn (from August till January) and spring (from January/February till July) seasons as short cycle in order to avoid from the cold temperatures in winter time or as long cycle starting from September/October till July.

The sizes of the greenhouses are small in Turkey and they are mostly family business greenhouses. 64% of glass greenhouses and 56% of plastic greenhgouses are 0.1-0.2 hectar range. 91% of glass greenhouses and 64% plastic greenhouses are less than 0.3 ha in Turkey (Sevgican *et al.*, 2000).

Tomatoes were growed in 57% of total gerenhouse area in 2015. 77% of greenhouse tomatoes was produced in plastic greenhouses. Greenhouse tomato production increased 64% in 2005-2014 period (Table 1).

In Turkey, greenhouse production is generally located on the coastal regions. Beside the Mediterranean coastal line, greenhouse practices are also expanded to Aegean, Marmara, Black Sea and South Eastern Anatolia regions. In 2015, about 81% of the greenhouse tomato production area in Turkey is concentrated in the Mediterranean region (Table 2).

Table 1. Greenhouse tomato growing areas and production in Turkey

Years	Growing area (ha)			Production (ton)		
	Glass greenhouse (1)	Plastic greenhouse (2)	Total (1+2)	Glass greenhouse (3)	Plastic greenhouse (4)	Total (3+4)
2006	5,293	11,065	16,358	751,196	1,150,055	1,901,251
2007	5,331	11,477	16,808	779,183	1,387,532	2,166,715
2008	6,122	12,482	18,604	831,188	1,292,792	2,123,980
2009	5,834	13,829	19,663	842,132	1,578,853	2,420,985
2010	5,885	14,664	20,549	818,074	1,769,075	2,587,149
2011	5,348	15,874	21,222	737,453	2,006,602	2,744,055
2012	5,455	17,004	22,459	705,618	2,132,540	2,838,158
2013	5,424	17,156	22,580	739,738	2,215,191	2,954,929
2014	5,473	16,261	21,734	758,951	2,261,956	3,020,907
2015	5,136	17,626	22,762	713,610	2,396,282	3,109,892

Source: TurkStat (http://www.tuik.gov.tr)

Table 2. Greenhouse tomato growing according to regions in Turkey

Regions	Years	Growing area (ha)	%	Production (ton)	%
Mediterranean	2006	13,295	81.27	1,555,480	81.81
	2015	18,534	81.43	2,477,264	79.65
Aegean	2006	2,848	17.41	318,837	16.77
	2015	3,761	16.52	570,938	18.36
East Marmara	2006	74	0.45	11,213	0.59
	2015	202	0.89	20,715	0.67
South Eastern Anatolia	2006	11	0.07	893	0.05
	2015	106	0.47	15,261	0.49
Middle Anatolia	2006	3	0.02	367	0.02
	2015	33	0.14	7,178	0.23
Others	2006	127	0.78	14,461	0.76
	2015	126	0.55	18,536	0.60
Total	2006	16,358	100.00	1,901,251	100.00
	2015	22,762	100.00	3,109,892	100.00

Source: TurkStat (http://www.tuik.gov.tr)

The majority of the greenhouses in the Mediterranean region are located in province of Antalya. Kumluca district of Antalya is an important plain with its intensive agricultural activities employing greenhouses. This climate zone allows unheated greenhouse production most of the time due to abundant solar radiation during the winter season. The greenhouse area usually heated only for frost prevention (Kacira *et al*., 2004).

MARKETING STRUCTURE OF GREENHOUSE TOMATOES IN TURKEY

Wholesale markets are the main point of sale for tomatoes in Turkey. Growers deliver their products to brokers in the wholesale centres who in turn sell the product on the grower's behalf. In this marketing system, growers do not have any marketing power (Yucel Engindeniz and Ucar, 2015). Sometimes it is possible to sell products outside this structure, e.g., selling of limited products directly to consumers by growers, exporting of products, and selling by cooperative (Figure 1).

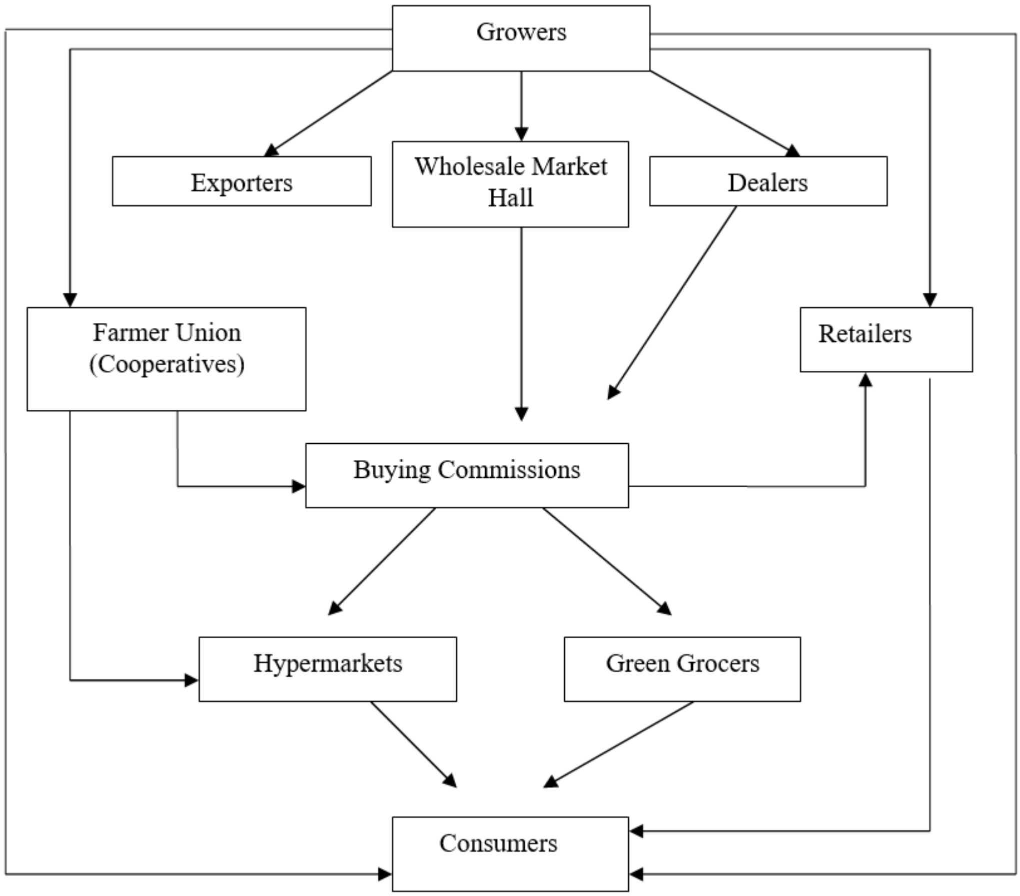


Figure 1. Marketing channels of fresh greenhouse tomato in Turkey
Source: Engindeniz *et al.*, 2009.

Fresh tomato prices in domestic markets of Turkey are presented in Table 3. Tomato production increases can decrease domestic prices and growers are disadvantaged as a result for some years. Grower prices varied between 0.26-0.51 €/kg in 2006-2015 period. In the same period, consumer prices varied between 0.55-0.84 €/kg. Even though greenhouse-grown tomatoes command a higher price than field-grown tomatoes, consumers are willing to pay for the higher quality and seasonal availability the greenhouse tomatoes offer.

Table 3. Fresh tomato prices in domestic market of Turkey (€/kg)

Years	Grower pice (1)	Retail price for consumer (2)	Marketing margin (2-1)
2006	0.26	0.55	0.29
2007	0.33	0.84	0.51
2008	0.29	0.58	0.29
2009	0.29	0.59	0.30
2010	0.51	0.84	0.33
2011	0.33	0.61	0.28
2012	0.35	0.70	0.35
2013	0.30	0.80	0.50
2014	0.27	0.69	0.42
2015	0.28	0.74	0.46

Source: TurkStat (http://www.tuik.gov.tr)

The majority of the tomatoes produced are consumed domestically in Turkey. But, Turkey also exports some tomatoes to countries such as Russia, Germany, Bulgaria, Romania, Irak, United Kingdom, and USA. In 2015, Turkey exported to different countries 544,357 tons tomato. Average tomato export of Turkey was 508,919 tons in 2006-2015 period. Total tomato export value of Turkey was 331.17 million € in 2015 and tomato export value formed 62.16% of total fresh vegetable export value of Turkey (Table 4).

Table 4. Fresh tomato export of Turkey

Years	Tomato export quantity (ton)	Tomato export value (1,000 €)	Total fresh vegetable export value (1,000 €)	Share of tomato in total fresh vegetable export value (%)
2006	306,033	140,346	270,498	51.88
2007	394,235	231,332	403,696	57.30
2008	483,282	292,060	460,323	63.45
2009	565,325	310,766	470,128	66.10
2010	574,724	365,648	544,762	67.12
2011	581,436	316,221	495,059	63.87
2012	561,997	313,760	500,471	62.69
2013	486,126	295,240	520,341	56.74
2014	591,675	323,388	533,808	60.58
2015	544,357	331,169	532,753	62.16

Source: AKIB (Mediterranean Exporter Associations General Secretariat) (http://www.akib.org.tr)

COST ITEMS OF GREENHOUSE TOMATO GROWING IN TURKEY

In Turkey, glass and plastic greenhouses which are generally used for tomato production are constructed without any project or planning criteria. For these kinds of greenhouses, the entrepreneurs go to local blacksmiths and construct greenhouses without taking any standard or engineering information into account (Emekli *et al*., 2010). High-technology greenhouses are generally built with galvanised iron support structure and glass or plastic as covering material. More advanced growing technologies, including hydroponics, are used in those greenhouse (Tuzel, 2003).
Approximate unit installation cost for plastic and glass greenhouses were 35 €/m^2 and 53 €/m^2 in 2015 according to data of The Turkish Ministry of Environment and Urbanisation. In 2006-2015 period, unit installation cost of plastic greenhouses varied between 29 and 36 €/m^2. But, unit installation cost of glass greenhouses varied between 49 and 62 €/m^2. Unit installation cost of plastic greenhouses vary between 24 and 31 €/m2 according to 2015 data of commercial greenhouse manufacturer in Turkey (Serprofil, 2017).
One of the purposes in a greenhouse enterprise is to provide and maintain the environment that will result in an optimum crop production or maximum profit (Emekli *et al*., 2010). The likely reason for the higher growth rate of the plastic greenhouses is due to their lower investment costs per unit area compared to glass houses (Yilmaz *et al*., 2005).
Greenhouse production is a highly intensive enterprise requiring substantial labor and capital inputs. Because of this, potential growers should carefully consider all of the factors necessary for better crop yields, higher labor productivity, energy efficiency and a successful enterprise (Kacira *et al*., 2004).
Greenhouse tomato enterprise budgets contain two types of costs, variable and fixed. The variable costs include costs for labor, fertilizer, seed, electricity, certification, marketing, transportation, and interest on total variable cost. Variable costs are calculated by using current input prices and labor costs. The fixed costs include interest on total initial investment, annual initial investment costs, costs, administrative costs, and land rent. Total costs are subtracted from total gross return to calculate the net return (Engindeniz and Tuzel, 2002; Engindeniz *et al*., 2009).
The results of conducted studies in different regions on unit cost of greenhouse tomatoes in Turkey are presented in Table 5. Unit cost for conventional growing vary beetween 0.21 and 0.29 €/kg according to growing period.

Table 5. Results of previous studies on unit cost of greenhouse tomatoes in Turkey

Region	Production period	Cover type	Yield (kg/m^2)	Total cost (€/m^2)	Unit cost (€/kg)	Grower price (€/kg)	Reference
Izmir	Autumn	Plastic	11.31	3.31	0.29	0.35	Engindeniz, 2003
Izmir	Autumn (organic)	Plastic	7.29	3.46	0.47	0.67	Engindeniz and Tuzel, 2002
Mugla	One-Crop	Glass	15.93	3.70	0.23	0.26	Engindeniz *et al*., 2009
Mersin	Spring	Plastic	9.00	2.31	0.26	0.64	Rad and Yarsi, 2005
Antalya	Spring	Glass	14.75	3.30	0.22	0.24	Engindeniz *et al*., 2009
Antalya	One crop	Glass	22.56	4.78	0.21	0.32	Karaman and Yilmaz, 2006

GOVERNMENT SUPPORTS FOR GREENHOUSE TOMATO GROWING IN TURKEY

There is no direct government support policy for greenhouse tomato production in Turkey. However, growers benefit from the general support policies pursued in agricultural production. Greenhouse procedures are supported by agricultural investments, and input and export support within the context of government support policies. The Agricultural Bank of Turkey has provided investment and special project credits to support growers. Furthermore, short-term credit support is provided to growers by Agriculture Credit Cooperatives. In 2006-2015 period, subsidy practises by the Turkish Ministry of Food, Agriculture, and Livestock for growers are presented in Table 6. Direct income support removed in 2008. Further, Turkish Lira depreciated against US dollar in 2015.

Table 6. Subsidy practises for greenhouse growers in Turkey

Subsidy practises	Unit subsidy (€/ha)		
	2006	2010	2015
Soil analysis	5.55	12.53	8.28
Fuel	9.99	16.29	16.06
Fertilizer	7.94	21.30	21.86
Beneficial insects for biological control	-	-	1,523.59
Bumble bees (*)	-	25.06	19.87
Organic production	16.65	125.32	231.85
Good agricultural practices	-	401.00	496.82
Direct income support	55.50	-	-

(*) Subsidy is given per Bumble bee colony.
Source: The Turkish Ministry of Food, Agriculture, and Livestock (http://www.tarim.gov.tr)

CONCLUSION

Turkey possesses some advantages in terms of climatic conditions, geothermal sources, cheap labor and as a consequence the chance exportation is increased. But, there are several problem in greenhouse production of Turkey. Over fragmented structure of production areas and small farm size is one of the most important problem. Thus production is realized in low-tech greenhouses resulting in intensive use of chemicals. Further, growers are also facing serious problems such as declining crop prices, price fluctuations based on over-supply, a poor market system and sales uncertainty, and a lack of grower cooperatives.

Greenhouse tomato production is generally carried out by small family-owned enterprises in Turkey. The number of large enterprises using modern technology is rather limited. Therefore, effective support policies should be practised for greenhouse tomato production. Greenhouse tomato production requires more intensive inputs than field crop cucumbers. Growers should be trained about effects of overdose pesticide and fertilizer use the environment and soil. Further, integrated pest management applications should be spreaded among growers to protect the environment and human health. Growers are also facing serious problems such as declining crop prices, price fluctuations based on over-supply, a poor market system and sales uncertainty, and a lack of grower cooperatives. Within the context of the organisation, cooperatives and growers' associations could play an important role in solving, in particular, the marketing problems of growers.

According to this study, tomato production in greenhouses may be profitable. But growers should gather all the economic data about the greenhouse tomato production, and market conditions before building a greenhouse. Also growers should make investigations on other greenhouse enterprises and determine if greenhouse tomato can be profitable. Although, cost and return estimates are believed to be typical and realistic, individual growers should adjust these values to their own specific situations and circumstances.

REFERENCES

AKIB (Mediterranean Exporters Union). (2017): Foreign Trade Data, http://www.akib.org.tr, Accessed: July 2017.

Emekli, N. Y., Kendirli, B., Kurunc, A. (2010): Structural analysis and functional characteristics of greenhouses in the Mediterranean Region of Turkey, African Journal of Biotechnology, Vol.9, No.21; pp. 3131-3139.

Engindeniz, S., Tuzel, Y. (2002): The Economic analysis of organic greenhouse tomato production: a case study for Turkey, Agro Food Industry Hi-Tech, Vol.13, No.5; pp.26-30.

Engindeniz, S. (2003): An economic analysis of growing conventional greenhouse tomatoes in Turkey, Practical Hydroponics and Greenhouses, Vol. 69, No.2003; pp.63-66.

Engindeniz, S., Yilmaz, I., Durmusoglu, E., Yagmur, B., Eltez, R. Z., Demirtas, B., Yucel Engindeniz, D., Tatarhan, A. H. (2009): Analysis of input usage with respect to improve of safe vegetable production in greenhouses, Publication of the Chamber of Agricultural Engineers, Izmir, pp.1-312.

Kacira, M., Sase, S., Kacira, O., Okushima, L., Ishi, M., Kowata, H., Moriyama, H. (2004): Status of greenhouse production in Turkey: focusing on vegetable and floriculture production, Journal of Agricultural Meteorology, Vol. 60, No.2; pp.115-122.

Karaman, S., Yilmaz, I. (2006): The Effects of Using of Bumble Bees for Pollination on inputs and profitability in glasshouse tomatoes production, Anadolu, Vol.16, No.2; pp.90-109.

Rad, S., Yarsi, G. (2005): Economic Performance and Unit Production Cost in Greenhouse Farms Producing Tomatoes in Silifke, Journal of Agricultural Sciences, Vol.11, No. 1; pp. 26-33.

Serprofil. (2017): Greenhouse projects and prices, http://www.serprofil.com, Accessed: July 2017.

Sevgican A., Tüzel, Y., Gul, A., Eltez, R. Z. (2000): Protected cultivation in Turkey. In: Proceedings of the Fifth Technical Congress of Chamber of Turkish Agricultural Engineers, January 17-21, 2000, Ankara, pp.679-707.

The Turkish Ministry of Environment and Urbanisation. (2017): Approximate unit costs for buildings, http://www.csb.gov.tr, Accessed: July 2017.

The Turkish Ministry of Food, Agriculture, and Animal Husbandry. (2017): Agricultural Supports, http://www.tarim.gov.tr, Accessed: July 2017.

Turkish Statistical Institute (TurkStat). (2017): Agricultural Statistics, http://www.tuik.gov.tr, Accessed: July 2017.

Tuzel, Y. (2003): Protected cultivation in Turkey, 5th FAO Regional Working Group Meeting on Greenhouse Crop Production in the Mediterranean Region, 10-13 November, 2003, Nicosia, Cyprus.

Tuzel, Y., Leonardi, C. (2009): Protected cultivation in Mediterranean Region: trends and needs, Journal of Agricultural Faculty of Ege University, Vol. 46, No.3; pp. 215-223.

Tuzel, Y., Gul, A., Dasgan, H. Y., Oztekin, G. B., Engindeniz, S., Boyaci, H. F. (2015): Changes and New Searches in Protected Cultivation, In: Proceedings of the Eighth Technical Congress of Chamber of Turkish Agricultural Engineers, 12-16 January, 2015, Ankara, pp.685-709.

Yilmaz, I., Sayin, C., Ozkan, B. (2005): Turkish Greenhouse Industry: Past, Present, and Future, New Zealand Journal of Crop and Horticultural Science, Vol. 33, No.3; pp. 233-240.

Yucel Engindeniz, D, Ucar, K. (2015): Economic aspects of greenhouse tomato production in Turkey, 26th International Scientific-Expert Conference on Agriculture and Food Industry, Sarajevo/Bosnia and Herzegovina, pp.377-381.

Indeks autora / Authors' index

UPUTSTVO ZA OBJAVLJIVANJE RADOVA

Radovi Poljoprivredno-prehrambenog fakulteta Univerziteta u Sarajevu (Radovi) su godišnjak u kojem se objavljuju naučni, izuzetno i stručni radovi, te izvodi iz doktorskih i magistarskih teza odbranjenih na Poljoprivredno-prehrambenom fakultetu Univerziteta u Sarajevu (Fakultet).

Radovi imaju karakter naučnog časopisa i kao takvi podliježu propozicijama za takve publikacije. Od broja 52 Radovi su indeksirani kod CAB Publishing - UK.

Članci za objavljivanje se klasificiraju, po preporuci UNESCO-a, u ove kategorije: naučni radovi, prethodna saopštenja, pregledni i stručni radovi. Autori predlažu kategoriju za svoje članke, recenzenti preporučuju, a konačnu odluku o kategorizaciji donosi Redakcija Radova. Naučni radovi sadrže rezultate izvornih istraživanja. Njihov sadržaj treba da bude izložen tako da se eksperiment može reprodukovati i provjeriti tačnost analiza i zaključaka. Prethodna sopštenja sadrže one značajne naučne rezultate, koji zahtijevaju hitno objavljivanje. Ova istraživanja mogu biti vremenski kraća od uobičajenih. Pregledni radovi sadrže pregled neke problematike na osnovu već publikovanih tekstova, koja se u pregledu analizira i diskutuje. Stručni radovi su korisni prilozi iz područja struke, koji ne predstavljaju izvorna istraživanja.

Članci se pišu na bosanskom, srpskom, hrvatskom ili engleskom jeziku. Na početku rada treba pisati naziv rada (velikim slovima) na maternjem i na engleskom jeziku, a nakon toga ime (imena) autora. Naziv radne organizacije autora upisuje se u fusnotu (Arial 7). Ispod imena autora obavezno se upisuje i kategorija rada.

U časopisu se publikuju radovi iz oblasti: poljoprivredna biljna proizvodnja, animalna proizvodnja, prehrambene tehnologije i održivi razvoj agrosektora i ruralnih područja.

Poželjno je da članci naučnog karaktera imaju uobičajenu strukturu naučnog rada i to: rezime (na bosanskom, srpskom i hrvatskom), summary na engleskom jeziku, uvod, pregled literature (može se dati i u uvodu), materijal i metode rada, rezultati istraživanja, diskusija (može biti objedinjeno sa rezultatima istraživanja), zaključci, literatura. Rezime i summary na našim jezicima i engleskom jeziku mogu imati maksimalno 200 riječi, uz obavezno upisivanje ključnih riječi. U spisku literature daju se samo autori i radovi koji se spominju u tekstu. Imena autora u tekstu pišu se spacionirano (Home→Font→Spacing→Expanded). Latinska imena biljaka, životinja i mikroorganizama treba (osim imena autora) pisati kurzivom. Tabele, grafikoni i slike moraju imati svoj naziv, a ako ih je više i broj. Broj i naziv tabele pišu se u istom redu, iznad tabele, dok se broj i naziv grafikona, crteža i slika pišu ispod tih priloga. U tabelama, grafikonima i slikama naslove, zaglavlja i objašnjenja poželjno je dati i na stranom jeziku. Grafikone i crteže treba raditi isključivo u crno-bijeloj tehnici. Tabele uokviriti linijama debljine 1/2 pt, bez sjenčenja pojedinih ćelija, ili redova i kolona. Slike i grafički prikazi treba da budu besprijekorne izrade radi kvalitetne reprodukcije u knjizi.

Radovi, po pravilu, ne treba da budu duži (sa prilozima) od 12 kucanih stranica. Izvodi iz magistarskih teza mogu biti dugi do 15, a iz doktorata do 25 kucanih stranica.

Za sadržaj članka odgovara autor. Članci se prije objavljivanja po "*double blind*" principu recenziraju od strane dva nezavisna recenzenta. Redakcija, uz konsultovanje sa autorima, zadržava pravo manjih redaktorskih i jezičkih korektura u člancima.

Autor dostavlja Redakciji rukopis putem e-maila uređen prema uputstvima za pisanje radova. Prilikom slanja radova Redakciji obavezno je naznačiti kontakt adresu i e-mail adresu u posebnom dokumentu.

Svi prispjeli rukopisi će biti podvrgnuti inicijalnoj provjeri u pogledu zadovoljenja kriterija oblasti iz kojih časopis objavljuje radove i tehničke pripreme rukopisa u skladu sa uputstvima autorima.

Podneseni rukopis nakon inicijalne provjere od strane Redakcije može biti odbijen bez recenzija, ako uredništvo ocijeni da nije u skladu s pravilima časopisa. Autoru će u roku od 20 dana biti upućena informacija o inicijalnom prihvatanju rada ili razlozima za njegovo neprihvatanje. Po prijemu informacije o inicijalnom prihvatanju rada, autor je dužan izvršiti uplatu prema uputi i dostaviti skeniranu uplatnicu na adresu Redakcije. Nakon izvršene uplate rukopis se šalje na recenziju.

Po završetku postupka recenziranja koji, u pravilu, ne bi trebao trajati duže od tri mjeseca Redakcija, na osnovu konačnih preporuka recenzenata, donosi odluku o objavljivanju, odnosno neobjavljivanju rada. O svojoj odluci Redakcija informiše autora, uz informaciju o broju i terminu izlaska časopisa u kojem će rad prihvaćen za objavljivanje biti štampan.

Elektronsku verziju rada treba pripremiti u Wordu u formatu stranica 170 x 240 mm, sa slijedećim veličinama margina: gornja i donja 2,2 cm, lijeva 2,0 cm, a desna 1,5 cm, te formatirati parne i neparne stranice. Isključivo koristiti font Times New Roman, veličina 11, dok za fusnote treba koristiti font Arial, veličina 7. Tekst treba da je obostrano poravnat. Nazive poglavlja u radu treba pisati velikim slovima, boldirano i sa srednjim poravnanjem, te jednim redom razmaka od teksta.

Prilikom formatiranja članka ne treba uređivati zaglavlje i podnožje članka (Header and Footer) niti numerisati stranice.

Autorima kojima engleski jezik nije maternji, strogo se preporučuje da obezbijede profesionalnu korekturu teksta koji će biti recenziran. Prilikom pisanja na engleskom jeziku treba koristiti jasne engleske izraze bez žargona i izbjegavati duge rečenice. Strogo se preporučuje da autor prije slanja rukopisa izvrši provjeru teksta na engleskom jeziku koristeći opciju „spelling and grammar“. Prihvatljivi su i britanski i američki „spelling“, ali on mora biti konzistentan u cijelom tekstu rada na engleskom jeziku.

Prije pisanja članaka za Radove, poželjno je da autori pogledaju formu radova već objavljenih u jednom od zadnjih brojeva ili da na web stranici: www.ppf.unsa.ba, pronađu uputstva sa primjerom pravilno uređenog članka.

Za radove čiji je prvi autor iz Bosne i Hercegovine nakon inicijalnog prihvatanja rukopisa treba uplatiti iznos od 100 KM na žiro račun Fakulteta. Za radove čiji je prvi autor izvan Bosne i Hercegovine nakon incijalnog prihvatanja rukopisa treba uplatiti 100 € na devizni račun Fakulteta koji se nalazi na podlistku Radova. Uplata je obavezna bez obzira na konačnu odluku o objavljivanju, odnosno neobjavljivanju rada.

TRANSAKCIJSKI RAČUN BR: 3389002208130126 UNICREDIT BANK

IBAN: BA393389104801155558 UNICREDIT BANK

SWIFT: UNCRBA 22

Pridržavajući se ovih uputstava, autori ne samo da olakšavaju posao Redakciji, nego i doprinose da njihovi radovi budu pregledniji i kvalitetniji. Više informacija, autori mogu dobiti obraćanjem Redakciji na e-mail: radovi@ppf.unsa.ba

Odštampani Radovi se dopremaju u biblioteku Fakulteta, odakle se vrši slanje Radova u AGRIS i CAB Publishing – UK u pisanoj i elektronskoj verziji, odnosno svaki objavljeni broj Radova posebno u PDF i Word formatu. Biblioteka vrši korespondenciju i razmjenu Radova s drugim institucijama u zemlji i inostranstvu, te šalje sveske Radova autorima i koautorima.

Redakcija

INSTRUCTION FOR PUBLISHING PAPERS

“Radovi Poljoprivredno-prehrambenog fakulteta Univerziteta u Sarajevu” (“Works of the Faculty of Agriculture and Food Sciences of University of Sarajevo), hereinafter: “Radovi” (the “Works”) is an almanac in which (original) scientific papers, exceptionally professional papers, and also some excerpts from doctoral/PhD or master theses defended at the Faculty of Agriculture and Food Sciences (the Faculty) of University of Sarajevo (Univerzitet u Sarajevu) are published.

“Radovi” (the “Works”) has a character of scientific magazine and, as such, is subject to the propositions for such publications. Since its issue no. 52, “Radovi” (the “Works”) has been indexed at CAB Publishing - UK.

Articles for publishing are classified, according to the recommendation by the UNESCO, into these categories: (original) scientific papers, previous statements, (scientific) review and professional papers. The authors propose the category for their articles, critics recommend it and final decision on their categorisation is made by the Editorial Board of the “Radovi” (the “Works”). (Original) Scientific papers contain results of authentic researches. Their content should be presented in such a manner that an experiment may reproduce and verify accuracy of the analyses and conclusions. Previous statements contain those significant scientific results that require urgent publishing. These researches can be shorter in time than the usual ones. (Scientific) Review papers contain an outline of certain problems on the basis of previously published texts that are analysed and discussed about in the review. Professional papers are useful articles/works from the professional domain that do not present authentic researches.

Articles are written in one of the three official languages of BiH (Bosnian/Serbian/Croatian) or English. The title of the paper should be written at the beginning of the paper (in capital letters) in one’s mother tongue and in English and after that the author’s name (authors’ names). The author’s working organisation name is written in the footnote (Arial 7). It is mandatory to write out the category of the paper below the author’s name as well.

Papers from the areas of: agricultural plant production, animal production, food technologies and sustainable development of agro-sector and rural areas are published in the journal.

It is desirable that articles of scientific character have common structure of a scientific paper, namely: summary in one of the three official languages of BiH (Bosnian/Serbian/Croatian), summary in English, introduction, references (may be given in the introduction, too), material and methods, results of research, discussion (may be integrated with results of research), conclusions, bibliography. Summary in one of the three official languages of BiH (Bosnian/Serbian/Croatian), and summary in English respectively may have maximum 200 words, with mandatory enlisting of the key words. In the list of bibliography, only authors and papers that are mentioned in the text are given. The authors’ names in the text are written with expanded spacing (Home→Font→Spacing→Expanded). Latin names of plants, animals and micro-organisms should be written in italics. Tables, graphs and pictures must have their title and also if they are numerous, their number. The number and the title of the table are written in the same row above the table while the number and the title of the graph, drawing and pictures are written below them. It is desirable to give titles, headings and explanations in the tables, graphs and pictures in the foreign language, too. Graphs and drawings should be done exclusively in black-and-white technique. Tables should be framed in lines of thickness of 1/2 pt, without shading of individual cells or rows and columns. Pictures and graphic illustrations should be done impeccably in order to be top-quality reproduced in the book.

Papers, as a rule, should not be longer than 12 typed pages (with appendices). Excerpts from master theses may be even up to 15 pages, and from doctoral/PhD theses up to 25 typed pages.

The author is responsible for the contents of the article. Prior to their publishing, articles are reviewed under "*double blind*" principle by two independent reviewers. The Editorial Board, in consultations with the authors, reserves the right to minor editorial and linguistic corrections in the articles.

The author submits one’s manuscript to the Editorial Board by the means of e-mail edited according to the instructions for writing papers. On the occasion of sending papers to the Editorial Board it is obligatory to indicate the contact address and e-mail address in a separate document.

All the submitted manuscripts shall be subject to initial check in terms of meeting the criteria of the field which the magazine publishes papers from as well as technical preparation of the manuscript in accordance with the instruction to the authors.

Upon the initial check by the Editor, the submitted manuscript may be rejected without review if the Editor evaluates it is not in accordance with the journal’s rules. Within the term of 20 days, the notification shall be sent to the author about either initial acceptance of the paper or reasons for its rejection. Upon receiving the information on initial acceptance of the paper, the author is obliged to make payment according to the instruction and submit the scanned payment slip to the Editorial Board’s address. After the payment having been made, the manuscript is sent for review.

Upon completion of the reviewing procedure which, as a rule, should not last longer than three months, the Editorial Board, on the basis of final recommendations by reviewers, makes decision on publishing the pertinent paper or not. The Editorial Board then informs the author about their decision, in addition to the information on the issue and term of the article publishing which the paper accepted for publishing is going to be published in.

Electronic version of the paper should be prepared in Word, in page format of 170 x 240 mm, with the following size of margins: the upper and lower ones of 2,2 cm, the left one of 2,0 cm and the right one of 1,5 cm and then the even and odd pages formatted. The font of Times New Roman, size 11, is to be exclusively used, while for footnotes the font of Arial, size 7 should be used. The text should be aligned on both sides. The title of chapters in the paper should be written in capital letters, bold and with medium alignment as well as with one row of space from the text.

While formatting the article, neither header and footer nor page numbering should be arranged.

Authors whose mother tongue is not English are strongly recommended to provide professional corrections to the text that is going to be reviewed. While writing in English, clear English phrases without jargon should be used and long sentences should be avoided. Prior to sending the manuscript, it is strongly recommended for the author to carry out checking the text in English by using the option of "spelling and grammar". Both British and American spelling is acceptable but it must be consistent throughout the text of the paper in English.

Before writing articles for the "Radovi" (the "Works"), it is desirable that authors have a look at the form of papers having already been published in one of the recent issues or to find the instruction with an example of properly arranged article on the web site: www.ppf.unsa.ba.

For papers whose first author is from Bosnia and Herzegovina, upon initial acceptance of the manuscript one should pay the amount of 100 BAM to the bank giro account of the Faculty. For papers whose first author is outside Bosnia and Herzegovina, upon initial acceptance of the manuscript one should pay 100 € to the foreign currency account of the Faculty that is indicated in the sub-directory of the "Radovi" (the "Works"). The Payment is mandatory regardless the final decision on the publication.

TRANSACTION ACCOUNT No/TRANSAKCIJSKI RAČUN BR: 3389002208130126 UNICREDIT BANK

IBAN: BA393389104801155558 UNICREDIT BANK

SWIFT: UNCRBA 22

By adhering to these instructions, authors not only facilitate the job for the Editorial staff but also contribute to their papers to be presented better and in a more qualitative manner. Authors can get more information by contacting the Editorial Board at the e-mail: radovi@ppf.unsa.ba

Printed copies of the "Radovi" (the "Works") are delivered to the Faculty's Library where the "Radovi" (the "Works"), that is, each published issue of the "Radovi" (the

“Works”) is sent from, to AGRIS and CAB Publishing – UK, both in written and electronic version, separately in PDF and Word format. The Library carries out the correspondence and exchange of the “Radovi” (the “Works”) with other institutions in the country and abroad as well as sends volumes of the “Radovi” (the “Works”) to the authors and co-authors.

Editorial Board