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EFFECT OF TREATMENT WITH DIFFERENT CONCENTRATIONS OF INDOLE BUTYRIC ACID (IBA) ON THE ROOTING OF ELDER CUTTINGS (*Sambucus nigra* L.)*

Fikreta Behmen[1], Fazila Šehić*[1], Mersija Delić [1], Selma Haračić-Berbić[1]

Original scientific paper

Abstract

In this work, the influence of different concentrations of indole-butyric acid on the development of roots, one-year-old and two-year-old hardwood cuttings (*Sambucus nigra* L.) was investigated.
Both types of cuttings were treated with three different concentrations of indole-butyl acid in liquid form (500 ppm, 800 ppm, and 1000 ppm), including the control variant of one-year-old and two-year-old hardwood cuttings (without the application of hormones). The parameters examined at the end of the experiment were: the percentage of rooting of the cuttings depending on the hormone treatment, the length of the adventitious roots depending on the hormone treatment, and the fresh mass of the roots, also depending on the hormone treatment. The use of indole-butyl acid in a concentration of 800 ppm proved to be the best because it had the greatest effect on increasing the value of all tested parameters of the cuttings.

Key words: *cuttings, indole butyric acid, elderberry*

INTRODUCTION

Elderberry is a wild plant; it is not demanding in terms of cultivation, and it adapts to different conditions and habitats. It can most often be found at the edges of forests. It prefers deep, fertile, and moist soils, but it also tolerates drought well. It adapts well to all types of soil and tolerates pollution well, and for this reason, it is also suitable for growing in urban areas (Jemrić, 2007).
It most often grows as a wild crop, but it can also be grown as a cultivated crop. It is propagated by cuttings from older branches and cuttings from younger branches, but it can also be propagated from seeds. Elderberry is most often propagated vegetatively by green, semi-ripe, and mature cuttings. For cuttings, well-developed and healthy one-year cuttings are used, which are taken at the beginning of spring. These growths are cut into cuttings 10 to 12 cm long and 5 to 8 mm thick.

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When scarifying cuttings, agents are used that improve scarring, such as hormones. Auxins have a special influence on the scarring process. These are plant hormones that were among the first to be discovered. The most commonly used auxin is indole-acetic acid (IAA), which is a natural product of plant metabolism. Artificially created auxins are also very often used: indole butyric acid (IBA), which was used in this experiment. Auxins influence the elongation and division of cells, stimulating the growth of adventitious roots (rhizogenesis), inhibiting the growth of the main root, and the formation of adventitious buds. The effect of auxin is also in the apical (top) dominance of the tree: if the top of the stem is removed, the auxin will stimulate the development of side shoots, one of which takes over the dominant function (Hadžiabulić S., 2010).
This work aimed to examine the influence of different concentrations of indole butyric acid on the rooting of *Sambucus nigra* L. cuttings and to determine which concentration of indole butyric acid is optimal for the treatment of elderberry cuttings.

MATERIALS AND METHODS

In this work, mature cuttings were used that were taken from parent trees at the end of March 2023. The experiment was made immediately after taking the cuttings from the trees. This experiment consisted of the fact that the cuttings were treated with different concentrations of the hormone indole butyric acid. The hormone solution was made as follows: 100 mg or 0.1 g of IBA powder hormone was placed in a small flask and dissolved in 5 ml of ethanol, and then this content was transferred to a 100 ml measuring flask and then topped up with distilled water up to the mark. Solutions 0.8% and 0.5% were prepared in the same manner.
At the end of March 2023, 160 mature cuttings of the same length and thickness were taken from the parent trees. 20 pieces of cuttings each were treated with three different concentrations of IBA hormone, with the fact that within one concentration there were two varieties of cuttings (one-year cuttings elderberry and one-year cuttings with a two-year part), and the last 40 pieces of cuttings (20 one-year and 20 one-year with two-year partly) were not treated and they served as a control variant, i.e. cuttings without hormonal treatment.
The variants, that is, the concentrations of indole-butyric acid used in this experiment, were as follows:
1. IBA 500 ppm
2. IBA 800 ppm
3. IBA 1000 ppm
4. Control variant

The cuttings were taken from mother trees in the area of the Municipality of Hadžići. The lower part of the cutting was cut with a straight section just below the bud, and the upper part with a slight slope. The thickness of the cuttings was 5-7 mm. Planting was done early in the spring.
The application of the IBA hormone was done in such a way that before planting the cuttings in the substrate for rooting, they were immersed in the corresponding

previously prepared solution for thirty seconds. The substrate used for rooting was a mixture of sand and peat in a ratio of 75:25%.
The cuttings were placed in an upright position, with a distance of 5 to 7 cm, and two to three buds were located outside the surface of the substrate.
After sixty days, they were transplanted into plastic pots with a diameter of 11 cm, which were filled with a mixture of garden soil, sand and peat in a ratio of 50:25:25%.
After transplanting, further cultivation of young plants continued until the moment of testing the parameters of development, which was carried out one month after transplanting the cuttings into plastic pots.
The tested parameters of development were: the percentage of rooting of elderberry cuttings depending on the treatment of cuttings with IBA, the length of adventitious roots of ed cuttings depending on the treatment of cuttings with indole-butyric acid and the mass of fresh root matter of elderberry cuttings. The rooting percentage of the cuttings was determined by counting the scarified cuttings and converting that number into a percentage. The length of the adventitious root was determined by removing all the adventitious roots and measuring the longest adventitious root on a clean surface with a ruler. The mass of fresh root matter was determined by weighing on an analytical balance.
The obtained data were processed using standard statistical methods of analysis of variance (ANOVA) and multiple tests (LSD 0.05) with the use of Microsoft Excel 2003.
Based on the data analysis, the results were interpreted, and conclusions were drawn about the results of the research.

RESULTS AND DISCUSSION

By researching the influence of different concentrations of the IBA on scarring of one-year-old cuttings and one-year-old cuttings with a two-year part, it was determined that there is variability between the tested cuttings.
When regularly inspecting the cuttings, the first leaflets appeared already after two weeks. After two weeks of planting the cuttings, a difference could already be observed between the cuttings that were treated with the hormone and those that were not treated with the indole-butyric acid hormone.
Four months after the start of the experiment, the root system of the cuttings was checked, i.e. the percentage of scarring in all cuttings was determined, as well as the other parameters that were previously mentioned.

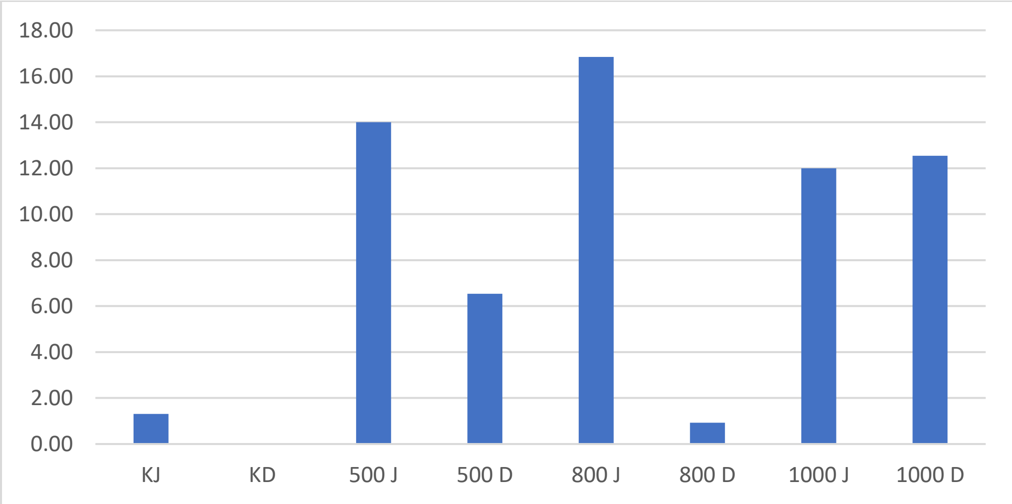
Table 1. The percentage of rooted cuttings elderberry depending on the treatment of the cuttings with indole-butyric acid

Variant	**Percentage of rooting**
One-year control variant (KJ)	15
One-year control variant with two-year part (KD)	0
500 ppm annually (500 J)	50
500 ppm one year with a two-year ppm part (500 D)	35
800 ppm annually (800 J)	65
800 ppm one year with a two-year part (800 D)	10
1000 ppm annually (1000 J)	60
1000 ppm one year with a two-year part (1000 D)	45

From the data presented in Table 1, it can be concluded that the one-year cuttings treated with a concentration of 800 ppm indole-butyric acid had the highest rooting percentage of 65%. In addition to this variant, two more stand out, elderberry one-year cuttings treated with a concentration of 500 ppm and a concentration of 1000 ppm of indole-butyric acid, whose percentage of rooting was 50% at a concentration of 500 ppm, and 60% at a concentration of 1000 ppm.

From the above data, it can be recomended to use one-year-old cuttings for rooting, and the reason for this finding is that these cuttings are younger and, accept the hormone better and root more easily.

The result of the analysis of the length of the adventitious root of the cuttings depending on the treatment of the cuttings with indole-butyric acid



Graph 1. Average values of the largest root lengths of the elderberry cuttings

From the data on graph 1, it can be seen that the length of the adventitious root was the largest in the variant where the one-year cuttings were treated with IBA at a concentration of 800 ppm.
To determine whether the influence of the application of indole-butyric acid on the length of the adventitious root of the cuttings was statistically significant, a variance analysis was performed, and the results of the analysis are shown in Table 2.

Table 2. Analysis of the variance of the influence of indole-butyric acid on the length of the adventitious root of the cutting

ANOVA

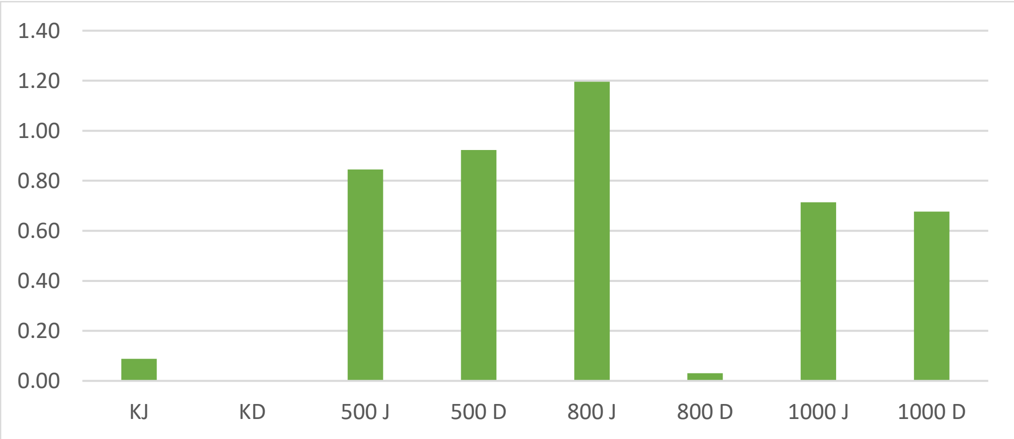
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4054.115	7	579.1593	11.52115	1.53E-10	2.106465
Within Groups	4825.846	96	50.26923			
Total	8879.962	103				

The analysis of variance showed that the length of the adventitious root of the cuttings was statistically significantly dependent on the treatment of the cuttings with indole-butyric acid.
The average root length of the cuttings obtained in that variety was statistically significantly higher than almost all other varieties tested. The only exception is the variant in which one-year cuttings are treated with indole-butyric acid at a concentration

of 500 ppm. The difference in the length of the adventitious root between the two varieties was not statistically justified.
Statistically, the lowest average value of the length of the adventitious roots of elderberry cuttings was achieved in the control variants, and in the variant where one-year-old elderberry cuttings with a two-year part were treated with indole-butyric acid at a concentration of 500 ppm.

Results of the analysis of the fresh mass of the adventitious root of the cuttings depending on the treatment of the cuttings with indole-butyric acid

The average values of the fresh mass of the adventitious root of the cuttings depending on the treatment of the cuttings with indole-butyric acid are shown in graph 2.



Graph 2. Average values of the fresh mass of the adventitious root of the cutting

From the data presented in the graph, it can be concluded that the highest mass of fresh root matter of the elderberry cuttings was determined in the variant where the one-year-old elderberry cuttings were treated with indole-butyric acid at a concentration of 800 ppm, and the lowest in the control variant. To determine the statistical significance of the influence of indole-butyric acid on the fresh mass of the roots of the cuttings, a variance analysis was performed, and the results of the analysis are shown in Table 3.

Table 3. Analysis of the variance of the influence of indole-butyric acid on the fresh root mass of the cuttings

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	7.361122	7	1.051589	3.625059	0.005481	2.312741
Within Groups	9.28284	32	0.290089			
Total	16.64396	39				

According to the data presented in Table 3, it can be concluded that the determined differences in the fresh mass of the roots of the cuttings between the applied variants were significant.

The best results for the tested parameters of cuttings treated with indole-butyric acid were obtained from one-year-old cuttings treated with a concentration of 800 ppm with indole-butyric acid.

In comparison with other literature sources (Murtić *et al*., 2015), elderberry cuttings treated with indole butyric acid at a concentration of 800 ppm or 0.8% showed a positive effect on increasing the values of all tested quality parameters of scallion cuttings.

According to Uzun *et al*. (2022) application of indole-butyric acid in lower concentrations showed a better effect compared to application in higher concentrations. The use of indole-butyric acid in a lower concentration had a positive effect on the rooting percentage of the cuttings, but also on the increase in the length of the adventitious roots.

Sönmezışık *et al*. (2021) claim that the length of the adventitious root was greater in one-year-old cuttings of elderberry than in two-year-old cuttings of elderberry treated with indole-3-acetic acid (IAA). This statement coincides with the results obtained in this work because we can conclude that the length of the adventitious root was the highest in the varieties where the one-year-old cuttings were treated with IBA.

Gudeva *et al*. (2017) believe that the use of plant hormones (IAA, IBA, and NAA) in concentrations of 100, 200, 300, and up to 1000 ppm has a favorable effect on the rooting of cuttings, but also on other species that are difficult to root, and that concentrations of 1000, 3000 and 5000 ppm did not have a visible effect on the speed of rooting of the cuttings, although they did affect morphological characteristics of new plants. They also claim that in addition to the use of these hormones, the rooting of cuttings also depends on several other factors such as climatic conditions, time of hormone application, growing medium, propagation system, etc.

CONCLUSIONS

Analyzing the obtained data, it was concluded that the application of the IBA in a concentration of 800 ppm is the most adequate for use on mature cuttings because this concentration has a positive effect on all quality parameters of cuttings. Analysis of variance determined that there are significant differences when using hormones at a concentration of 800 ppm and other concentrations.

In addition to the use of the indole-butyric acid hormone in a concentration of 800 ppm, the age of the cuttings is also of great importance, as could be seen in the results of the experiment, so it is recommended to use one-year-old elderberry cuttings for scarring, which of course are in optimal health.

From all of the above, it can be concluded that the results obtained in this paper can be connected with the results of the above-mentioned literature sources, given that the same views and conclusions were obtained: IBA in lower concentrations is optimal for the rooting of elderberry cuttings. In addition, one-year cuttings are the best for propagation because they are the easiest to root and have the longest adventitious roots.

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UTICAJ TRETIRANJA RAZLIČITIM KONCENTRACIJAMA INDOL-BUTERNE KISELINE (IBA) NA OŽILJAVANJE REZNICA ZOVE (*Sambucus nigra* L.)

Sažetak

U ovom radu je obavljeno istraživanje u kojem se opisuje uticaj različitih koncentracija indol-buterne kiseline na ožiljavanje jednogodišnjih reznica i jednogodišnjih reznica zove sa dvogodišnjim dijelom (*Sambucus nigra* L.). Obje varijante reznica zove su tretirane sa tri različite koncentracije indol-buterne kiseline u tečnoj formi (500 ppm, 800 ppm i 1000 ppm) uključujući i kontrolnu varijantu jednogodišnjih i jednogodišnjih reznica sa dvogodišnjim dijelom (bez primjene hormona).

Parametri koji su bili ispitivani na kraju završenog ogleda su bili: procenat ukorijenjavanja reznica zove u zavisnosti od tretmana reznica indol-buternom

kiselinom, analiza dužine adventivnog korijena reznica zove u zavisnosti od tretmana reznica indol-buternom kiselinom i rezultati analize svježe mase adventivnog korijena reznica zove u zavisnosti od tretmana reznica indol-buternom kiselinom.
Primjena indol-buterne kiseline u koncentraciji od 800 ppm se pokazala kao najbolja jer je imala najveći uticaj na povećanje vrijednosti svih ispitivanih parametara reznica zove.

Ključne riječi: *reznice, indol-buterna kiselina, zova*

UVOMETRIC PROPERTIES OF MERLOT AND VRANAC GRAPE VARIETIES DEPENDING ON PRUNING METHOD*

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Original scientific paper

Abstract

This work aimed to study the effect of pruning on the uvometric characteristics of the Merlot and Vranac wine varieties in the Trebinje region. The research was carried out in 2018, each variety had two variants of pruning, and each variant had three repetitions. The load levels of buds in this study were 11 (Variant I) and 9 (Variant II) buds per vine. The most important uvometric parameters of the tested varieties were measured. As an experimental factor, the variety, had a statistically significant influence on most of the investigated indicators, while pruning did not. The research carried out in Trebinje shows that both tested varieties can be successfully grown in the mentioned locality because the climatic conditions are suitable for their cultivation.

Keywords: *Merlot, Vranac, pruning, uvology*

INTRODUCTION

A fresh grape is destined for vinification, basically because of its characteristics. It can be overripe or slightly raisined or suffering from noble rot, provided that it may be crushed or pressed using normal winery procedures and that it is capable of undergoing a spontaneous alcoholic fermentation (OIV, 2022).

Pruning of grapevines is one of the most important viticultural measures during the formation of the vine's training system, enhancing yield and regular exploitation of the vineyard. Pruning can be done throughout the entire dormancy period, starting from leaf fall in autumn until the onset of vegetation in spring (Hellman, 2001; Himelrick and Dozier, 2013). Properly performed basic and supplementary pruning determines the growth, development, and fruiting of grapevines. The purpose of pruning is to achieve the maximum yield of high-quality grapes while allowing appropriate vegetative growth for the following growing season (Žunić and Matijašević, 2004). The quality of pruning affects the yield potential of the variety.

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Yield potential, as an economic characteristic, is a very important factor in production efficiency. It is expressed as grape yield per unit area, and, aside from the generative potential as a varietal trait, yield is also significantly influenced by environmental conditions and applied technology. The classification of varieties into low, medium, and high yield is primarily the result of their productive potential, as well as their adaptability to different cultivation systems, technological practices (load, fertilization, irrigation), and their adaptability to climatic and soil conditions of various regions (Maletić *et al*., 2008). In practice, the yield potential of a variety is determined by measuring the quantity of grapes harvested per unit area. This is the grape yield, defined by the number of clusters per vine, the average cluster weight, and the number of vines per unit area (Delić, 2015), all of which affect the cluster's and berry's morphological characteristics.

We determine measurable characteristics of the cluster and berry through uvometry (from the Latin uva – cluster). We measure cluster weight, cluster length, and width, berry count, and berry dimensions (length and width). Uvometry also provides information on berry size and basic shape. The berry index is calculated based on the length-to-width ratio of the berry. Uvometric studies are conducted at the full ripeness stage of the grapes, on a sample of no fewer than 10 clusters and 100 berries. The clusters and berries must be undamaged and collected in a specific manner to ensure sample representativeness (Maletić *et al*., 2008).

A very important ampelographic indicator of a grape variety is the mechanical composition of the cluster and berry. The mechanical composition of the cluster and berry is an ampelographic and technological property of each variety. Structural indicators of the cluster and berry, such as the share of skin and mesocarp in the berry, solid residue, and the proportion of berries within the cluster, significantly influence the technological properties of the cluster and, subsequently, the physicochemical properties of the wine (Intrieri *et al*., 2008). Mechanical analysis of the cluster and berry involves determining the quantitative and relative share of individual components in the total mass of the cluster or berry. Experience from numerous analyses shows that a grapevine cluster consists of 92-98% berries and 2–8% stems, while a berry consists of 5-11% skin, 2–5% seeds, and 80-90% flesh with juice. The absolute and relative values of the mechanical composition indicators differ among grapevine cultivars and under different growing conditions (Nastev, 1986). The share of skin, mesocarp, and seeds in the berry correlates with berry size. As berry size increases, so does the percentage of skin, mesocarp, and seeds in the berry (Matthews and Nuzzo, 2005).

The composition and structure of the cluster and berry indicate the proportion of individual structural elements within the cluster and berry, representing an ampelographic and technological trait of the variety (Matijašević, 2021).

Merlot and Vranac are wine grape varieties used for producing high-quality red wines. Merlot is a French variety popular worldwide, while Vranac is a Montenegrin variety widely cultivated across the Balkans.

This study aimed to determine the impact of pruning on the uvometric characteristics and key yield indicators of the wine grape varieties Merlot and Vranac under the agro-ecological conditions of Trebinje.

MATERIALS AND METHODS

Material: The wine grape varieties Merlot and Vranac, grafted onto *Berlandieri x Rupestris* Richter 110 rootstock. The experiment was set up in a production vineyard located in Trebinje during 2018, using a random selection method. The vineyard follows a trellis training system with a planting distance of 1.8 x 1.0 m. The training form for the vines is the Moser cordon. The vineyard is in full production, with standard agricultural practices applied. It was planted in 2008 (Merlot) and 2010 (Vranac) on karst soil. The climate is Mediterranean. Two pruning variants were studied: 11 buds per vine (Variant I) and 9 buds per vine (Variant II), each with three repetitions (6 vines per repetition). A total of 72 vines were included in the experiment (36 vines per variety). Laboratory analyses were conducted at the Faculty of Agriculture and Food Sciences, University of Sarajevo.
Methods for examining variety yield: The number of clusters per vine was determined by counting them during harvest, and the grape yield per vine was determined by measuring the weight of the grapes.
Methods for uvometric examination: The Prostoserdov method (Prostoserdov, 1964) was used. Mechanical analysis of the cluster was conducted on a representative sample of 10 clusters, harvested at full ripeness, for three repetitions within each pruning variant and for both grape varieties studied. An average sample of 100 berries was formed for the mechanical composition analysis by detaching berries from 10 clusters. The berries were visually classified into three categories: large, medium, and small. After counting, the percentage share of each berry category was calculated, and based on this, an average sample of 100 berries was created.
The uvometric analysis included the following parameters:
a) Cluster Composition:

- Cluster weight (g),
- Weight of the stem in the cluster (g),
- Weight of the berries in the cluster (g).

b) Berry Composition:

- Weight of 100 berries (g),
- Weight of the flesh in 100 berries (g),
- Weight of the skins from 100 berries (g),
- Weight of seeds in 100 berries (g),
- Number of seeds in 100 berries (pcs).

c) Cluster and Berry Structure:

- Percentage of stem,
- Percentage of skin,
- Percentage of seeds,

- Percentage of flesh,
- Skeletal,
- Solid residue,
- Structural index.

Results Analysis: A two-factor analysis of variance (ANOVA) was performed. The obtained parameters were processed using the statistical software SPSS (IBM SPSS Statistics). The standard error was determined using the LSD test at a significance level of 0.05.

Environmental Conditions

For the adequate development of grapevines, regular fruiting, and obtaining high yields of quality grapes, suitable environmental conditions are essential. The most important environmental conditions and factors are climatic, soil, and biotic. By selecting the right grape variety and rootstock, using quality planting material, choosing optimal planting spacing, and applying appropriate agricultural practices, humans can significantly contribute to successful viticulture production—but only within suitable ecological conditions (Burić, 1995).

For the analysis of climatic conditions, data from the Trebinje meteorological station were used. Trebinje has approximately 260 sunny days per year, making it one of the sunniest regions in the Balkans.

Table 1. Basic hydro-meteorological parameters in the Trebinje area

Parameter	2018	2001 - 2018
Mean annual air temperature (°C)	15.5	15.0
Mean vegetative air temperature (°C)	20.8	20.0
Absolute minimum air temperature (°C)	-6.5	-7.0
Absolute maximum air temperature (°C)	35.2	32.6
Precipitation during vegetation (mm)	637.7	711
Annual precipitation (mm)	1703.7	1706

The Trebinje region is characterized by warmer and drier summers, while winters tend to be more humid. During the study, air temperatures were higher compared to the long-term average, while the annual precipitation levels were consistent with the long-term average.

RESULTS AND DISCUSSION

The results of the analysis of yield, mechanical composition, and structure of the clusters and berries of the Merlot and Vranac varieties, depending on the pruning method, are presented in Tables 2, 3, 4, and 5.

Varietal Yield

The research results indicated that there was a statistically significant difference in grape yield per vine in Variant I between the Vranac and Merlot varieties, while no statistically significant difference was found in Variant II. Within the same variety, there was a statistically significant difference between the pruning variants (Table 2). The results of our study are following Delić *et al*. (2023).

Additionally, there was a statistically significant difference in the average number of clusters per vine between the Vranac variety (16.5) compared to the Merlot variety (20.4). However, no statistically significant difference was observed in the value of this parameter between the pruning variants within the same variety (Table 2).

Mechanical Analysis of the Cluster

The weight of the cluster was statistically significantly greater for the Vranac variety compared to the Merlot variety. A difference in cluster weight between the pruning variants within the Merlot variety was evident, while no such difference was observed in the Vranac variety (Table 2).

The average weight of the Merlot cluster in Sremski Karlovci was 106 g, while for the Vranac variety, it was 374 g (Cindrić *et al*., 2000). The average weight of the Vranac cluster in the Konjusi vineyard (Mostar) was 301.2 g (Sefo, 2009). According to the literature, the Merlot cluster is small, weighing around 100 to 130 g, depending on the clone, while the Vranac cluster is larger, weighing about 130 to 300 g (Žunić and Garić, 2010). In this study, the average weight of the Merlot cluster was 169.6 g, and for the Vranac variety, it was 230.5 g, which aligns with literature data and other research findings.

A statistically significant difference was also found in the average berry weight within the cluster between the Merlot (163.7 g) and Vranac (222.6 g) varieties. For the Merlot variety, a statistically significant difference was identified between the pruning variants, while for the Vranac variety, it was not (Table 2).

According to the literature, the average berry weight within the cluster for the Merlot variety is 80-100 g, while for the Vranac variety, it is 150-200 g (Žunić and Garić, 2010). Research conducted in the Konjusi vineyard on the Vranac variety reported this parameter at 292.31 g (Sefo, 2009).

The research results indicate that the variety and pruning variant did not have a statistically significant impact on the values of the stem weight parameter for the examined varieties. The average stem weight for the Merlot variety is 3-6 g, while for the Vranac variety, it is 7-10 g (Žunić and Garić, 2010). In the Konjusi vineyard, the value for the Vranac variety was reported as 9.22 g (Sefo, 2009). In this study, the average stem weight for the Merlot variety was 6.47 g, and for the Vranac variety, it was 7.96 g (Table 2).

According to the research results, it can be concluded that the variety and pruning variant did not have a statistically significant effect on the values of the cluster length (OIV descriptor Nº202) and cluster width (OIV descriptor Nº203) parameters for the examined varieties. The greatest cluster length was recorded for the Merlot variety in Variant II (16.81 cm), while the shortest was for the Vranac variety in Variant II (15.57 cm). The greatest width was found in the Vranac variety in Variant I (10.54 cm), and the smallest was in the Merlot variety in Variant II (9.53 cm) (Table 2).

Table 2. Average value of fertility of varieties and uvological analysis of clusters

Parameter	Average values	Pruning variant	Variety
Yield of grapes per vine (kg) $LSD_{0.05}$ 0.361	3.57^{b}	I	Merlot
	3.32^{c}	II	
	4.18^{a}	I	Vranac
	3.32^{c}	II	
Number of clusters per vine $LSD_{0.05}$ 3.799	19.52^{ab}	I	Merlot
	21.26^{a}	II	
	17.13^{b}	I	Vranac
	15.96^{b}	II	
Weight of cluster (g) $LSD_{0.05}$ 49.35	183.15^{b}	I	Merlot
	156.1^{c}	II	
	245.68^{a}	I	Vranac
	215.25^{ab}	II	
Weight berries in the cluster (g) $LSD_{0.05}$ 46.85	176.86^{b}	I	Merlot
	150.59^{c}	II	
	237.72^{a}	I	Vranac
	207.45^{ab}	II	
Weight of rachis (g)	6.3	I	Merlot
	5.47	II	
	7.96	I	Vranac
	7.8	II	
Length of cluster (cm)	16.45	I	Merlot
	16.81	II	
	16.06	I	Vranac
	15.57	II	
Width of cluster (cm)	10.18	I	Merlot
	9.53	II	
	10.54	I	Vranac
	9.7	II	

Length of berry (cm) $LSD_{0.05}$ 0.172	1.23^{c}	I	Merlot
	1.15^{c}	II	
	1.66^{a}	I	Vranac
	1.64^{ab}	II	
Width of berry (cm) $LSD_{0.05}$ 0.185	1.16^{c}	I	Merlot
	1.11^{c}	II	
	1.53^{ab}	I	Vranac
	1.59^{a}	II	

The values for the parameters berry length (OIV descriptor Nº220) and berry width (OIV descriptor Nº221) were statistically significant between the Merlot and Vranac varieties, while no significant differences were found between the pruning variants within the varieties. The average berry length for the Merlot variety was 1.19 cm, while for the Vranac variety, it was 1.65 cm. The average berry width for the Merlot variety was 1.135 cm, and for the Vranac variety, it was 1.56 cm (Table 2).

Mechanical Analysis of Berries
The research results indicate that there was a statistically significant difference in the value of the parameter for the mass of 100 berries between the Merlot and Vranac varieties, while no significant differences were found between the pruning variants within the varieties. The average mass of 100 berries for the Merlot variety was 132.85 g, while for the Vranac variety, it was 204.5 g (Table 3).

Table 3. Average value of uvological analysis of berry

Parameter	Average values	Pruning variant	Variety
Weight of 100 berries (g) $LSD_{0,05}$ 48,27	142^{bc}	I	Merlot
	123^{c}	II	
	184^{ab}	I	Vranac
	225^{a}	II	
Weight of pulp in 100 berries (g) $LSD_{0,05}$ 43,64	104^{b}	I	Merlot
	85^{c}	II	
	135.67^{ab}	I	Vranac
	175^{a}	II	
Weight of skin of 100 berries (g) $LSD_{0,05}$ 49,35	28.67^{c}	I	Merlot
	31.33^{ab}	II	
	40^{ab}	I	Vranac
	40.3^{a}	II	
Weight of seeds in 100 berries (g)	9.33	I	Merlot
	7.33	II	
	8.33	I	Vranac
	9.67	II	

Number of seeds in 100 berries	180	I	Merlot
	166.67	II	
	190	I	Vranac
	193.3	II	
	7.1	II	

The mass of 100 berries for the Merlot variety at the location of Poreč (Croatia), in the Plava Laguna vineyard, was reported at 124 g (Fazinić and Fazinić, 1985).
The research results also showed a statistically significant difference in the average value of the parameter for the mass of flesh in 100 berries between the Merlot (94.5 g) and Vranac (155.3 g) varieties, as well as between the pruning variants for the Merlot variety (Table 3).
Additionally, a statistically significant difference was found in the average value of the parameter for the mass of skins from 100 berries between the Merlot (30 g) and Vranac (40.15 g) varieties, as well as between the pruning variants for the Merlo variety (Table 3).
Differences in the values of the parameters seed mass and the number of seeds in 100 berries between the Merlot and Vranac varieties were not statistically significant. The highest seed mass was observed in the Vranac variety/variant II (9.62 g), while the lowest was in the Merlot variety/variant II (7.33 g). The number of seeds in 100 berries was greatest in the Vranac variety/variant II (193.3), and lowest in the Merlot variety/variant II (166.67) (Table 3).
The average seed mass in 100 berries for the Merlot variety ranges from 3.5 to 10 g, while for the Vranac variety, it ranges from 6 to 11 g (Žunić and Garić, 2010). The value of this parameter for the Merlot variety in studies conducted in Poreč was reported at 4 g (Fazinić and Fazinić, 1985).

Structure of the Cluster and Berry

The analysis of the structure of the cluster and berry was conducted using the Prostoserdov method (1964). The percentage of skin was consistent for both varieties. The percentage of skin was higher in the Vranac variety (19.5) compared to the Merlot variety (17.5). The Merlot variety had a higher average percentage of seeds (6) than the Vranac variety (4.25). The average values of the percentage of flesh in the berry were consistent for both varieties. The skeletal participation in the cluster was greater for the Vranac variety compared to the Merlot variety. The solid residue (the sum of the masses of stem, skin, and seeds in the cluster) was 26.98 for the Merlot variety and 26.8 for the Vranac variety. The structural index, which is the ratio of pulp mass in the cluster to solid residue, was also consistent for both varieties, ranging from 2.71 for the Merlot variety to 2.75 for the Vranac variety (Table 4). The values of structural indicators in this study align with other studies and literature data (Fazinić and Benčić, 1998; Žunić and Garić, 2010).

Table 4. Parameters of cluster structure according to Prostoserdov's method

Parameter	Average values	Pruning variant	Variety
Percentage of rachis (%)	3.44	I	Merlot
	3.5	II	
	3.2	I	Vranac
	3.6	II	
Percentage of skins (%)	19.4	I	Merlot
	15.6	II	
	21	I	Vranac
	17.3	II	
Percentage of seeds (%)	6.33	I	Merlot
	5.7	II	
	4.4	I	Vranac
	4.1	II	
Percentage of pulp (%)	70.83	I	Merlot
	75.2	II	
	71.4	I	Vranac
	75	II	
Skeletal	22.84	I	Merlot
	19.1	II	
	24.2	I	Vranac
	20.9	II	
Solid residue	29.17	I	Merlot
	24.8	II	
	28.6	I	Vranac
	25	II	
Structural index	2.42	I	Merlot
	3	II	
	2.5	I	Vranac
	3	II	

The uvometric indices calculated in this study included the berries index (number of grapes per 100 g of grapes) and the composition index (weight of flesh divided by the weight of skin plus seed) (Bora *et al*., 2014). The Merlot variety exhibited a significantly higher berries index (75.5) compared to the Vranac variety (49), while the composition index was higher for the Vranac variety (3.15) compared to the Merlot variety (2.45) (Table 5).

Table 5. Grape index of the wine grape varieties

Parameter	**Average values**	**Pruning variant**	**Variety**
Berries index	70	I	Merlot
	81	II	
	54	I	Vranac
	44	II	
Berries composition index	2,7	I	Merlot
	2.2	II	
	2.8	I	Vranac
	3.5	II	

CONCLUSIONS

The study of the impact of pruning type on the yield, mechanical, and structural composition of the clusters and berries of the grape varieties Merlot and Vranac was conducted in the Trebinje area during 2018. Based on the conducted research, it can be concluded that there was a statistically significant difference between the varieties in the values of most examined parameters, and these values were in accordance with many other studies.

In conclusion, it can be stated that the wine grape varieties Merlot and Vranac can be successfully cultivated in the Trebinje area. The climatic conditions are suitable for their cultivation. For most examined indicators, the variety had a statistically significant impact as a test factor, while pruning did not. This is due to the very small difference between the pruning variants (9 and 11 buds per vine).

This section should be concise, not repeating the text from above, and provide some recommendations for further studies, as well as present limitations of the study.

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UVOLOŠKE KARAKETRISTIKE SORTI VINOVE LOZE MERLOT I VRANAC U ZAVISNOSTI OD NAČINA REZIDBE

Sažetak

Cilj ovog rada je bio proučavanje uticaja rezidbe na uvološke karakteristike vinskih sorti Merlot i Vranac na području Trebinja. Istraživanje je obavljeno 2018 godine, svaka sorta je imala dvije varijante rezidbe, a svaka varijanta tri ponavljanja. Nivoi opterećenja

čokota u ovom istraživanju su bili 11 (Varijanta I) i 9 (Varijanta II) okaca po čokotu. Mjereni su najvažniji uvološki parametri ispitivanih sorti. Na većinu ispitivanih pokazatelja statistički značajan uticaj imala je sorta, kao ogledni faktor, dok rezidba nije.
Provedeno istraživanje na području Trebinja pokazuje da se obje ispitivane sorte mogu uspješno gajiti na navedenom lokalitetu jer su klimatski uslovi pogodni za njihovo gajenje.

Ključne riječi: *Merlot, Vranac, rezidba, uvologija*

APPLICATION OF NEW IMPROVED SOLUTIONS OF PREPARATIONS BASED ON ZEOLITH IN DIFFERENT PHASES OF PRODUCTION AT THE GRAPEVINE IN THE REPUBLIC OF NORTH MACEDONIA*

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Original scientific paper

Abstract

In the period from 2018 to 2023, we conducted research on the effects of the application of the innovative formula from the mineral zeolite on different stages of production in the grapevine in the Republic of North Macedonia.

The mineral zeolite originates from the mine excavations in Serbia. In the period from 2017 to 2018, a „improved formula" was made from the zeolite preparation with processing, it was crushed into a powder form, with a certain particle size for easy application in a dissolved state. This type of processed zeolite is registered as a soil improver and during that period it received an innovation award at a fair in Romania. We, in our country, continuously carry out tests using this preparation in several crops and it always gave positive results. In our study, the physico-chemical properties of this type of zeolite are shown, and then the results and the effect of the application on the vines are shown.

The main goal of applying this „ improved zeolite formula" was to reduce the use and uncontrolled use of pesticides in the fight to suppress diseases, pests, harmful weeds, etc. This is achieved in such a way that with the application of zeolite, which is environmentally friendly, the properties of the soil are improved, the plant makes better use of useful substances and water, in fact stronger, more resistant and immune plants to diseases, pests, harmful weeds, drought, frost are obtained. etc. At the same time, the use of pesticides is reduced, and the pollution of the environment is also reduced.

Keywords: *zeolite, improved formula, property improver, pesticides, grapevine*

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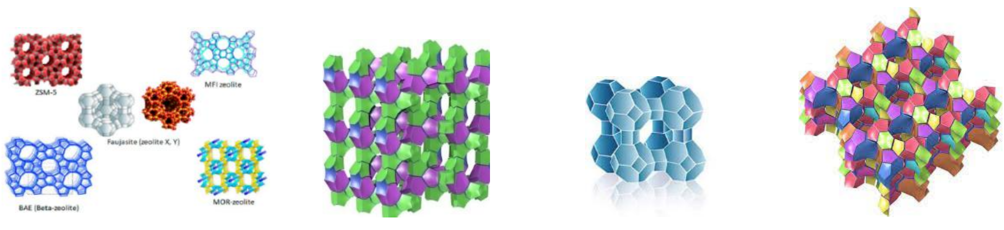
INTRODUCTION

The basic direction at the global level is healthy food and an unpolluted environment, but above all, it is necessary to produce food, that is, during the cultivation of agricultural crops, pollution of the environment should not occur. Therefore, organic agricultural production is increasingly being applied and solutions are being found on a global scale for less use of harmful substances in growing crops.
Zeolite is a mineral of volcanic origin and is separated in sedimentary deposits where there used to be lava. There are several natural types of zeolite (Baerlocher *et al.,* 2007). Zeolites used in agriculture are most often from the group of clinoptilolite and chabazite. It is most often extracted as pieces of stone, and then crushed and ground to a certain size. Zeolite is sold in granules and powder form. Depending on the deposit, according to the chemical composition, zeolites are complex hydrolyzed silicates of sodium, potassium and calcium (Flanigen *et al.,* 1980). They may also contain magnesium, aluminum, phosphorus, zinc, etc. The differences between zeolites actually consist in the percentage representation of these elements and in the size of the particles.
Zeolite is very suitable for organic agricultural production and its application reduces the use of synthetic harmful chemical preparations for the destruction of diseases, pests and weeds, because stronger and healthier plants with improved immunity are obtained. Its method of use is by introducing it into the soil and by foliar application. It is also an improver of soil properties, because it increases the availability of nutrients, water, air, regulates porosity, reduces acidity, etc.
Our innovative zeolite formula consists of a balanced percentage of elements and the method of further processing (crushing and finalization). The Terra powder preparation showed excellent results in improving the properties of soil and plants during testing in grapevines (Korunoska and Pešic, 2017).

MATERIALS AND METHODS

The tests in our paper were carried out in the period from 2018 to 2023 in grapevine plantations at individual producers in the Republic of Macedonia. Specifically, the effect of the application of zeolite with the marketing name - Terra powder on individual vines of the varieties Black Valandovo drenok, Muscat Italia, Chardonnay, Pinot noir was examined (Auerbach *et al.,* 2003). The following parameters were examined in the vines: soil pH, disease resistance and mass of removed mature shoots (kg/vine). Three vines were treated and 1 vine was left untreated for control. Treated vines are marked with T, and untreated vines are marked with U .
Four Treatments were carried out during the year, foliar - with the first treatment at the beginning of vegetation, the second treatment when there is a large green mass in summer, and the third before the end of vegetation (October - November) and the fourth is after the end of vegetation (December). If the soil is treated together with irrigation, it is necessary to make a previous detailed physicochemical analysis of it. But we performed an application with foliar treatment and treatment of the stem near the soil,

with a small manual pump (Gao *et al.,* 2024). Most often in viticulture, quantities of 1 to 3 tons per hectare are practiced, but for individual vines, the zeolite is applied in an amount of about 0.200 to 0.400 kg per vine. All measurements, analyses and comparisons were performed according to the usual tests of agrobiological properties according to the code system of OIV **Standards technical documents.** The statistical processing is Analysis of variance (ANOVA).
The zeolite in our tests comes from a mine near Kopaonik, R. Serbia and as such is a naturally very high-quality mineral. It is additionally processed by crushing it to microgranules and enriching it with certain elements that will further act by binding with other substances and form complex powder formulations. The processing was carried out in a small factory workshop in Skopje, R. N. Macedonia. The substance in powder easily turns into an agrogel and in a liquid state is easy to apply. Our „improved formula" has a very favorable structure, suitable grinding and good chemical composition. When applied as an improver of soil properties, it gives it a uniform porosity and moderate permeability. Treatment with zeolite in viticulture is not only completely harmless but also purifies the environment – water, air and soil.
Our innovative zeolite formulation was first tested at the Department of Pedology at the Faculty of Agricultural Sciences and Food in Skopje, and appropriate reports for recognition and approval have been submitted to the Ministry of Agriculture, Forestry and Water Economy and the Ministry of Environment and Spatial Planning. The preparation is listed under the proposed name GAIA - Terra powder, and table 1 shows the laboratory analyses of its chemical and physical properties.
Figures 1 to 4 show a model representation of the molecular composition of various types of zeolite. Figures 5 to 7 show mine excavation, different structure, innovative formula - Terra powder (Ivanković *et al.,* 2013).



1 **2** **3** **4**

Figure 1 – 4. Model representation of the molecular composition of various types of zeolite

Table 1. Results from properties improvement - Terra powder
* Unaccredited method

Parameter	Method	Received value	Declared value	Unit measure
Moisture	MKC ISO8190:1992	1.2	/	%
Mechanical composition (size in granules)	MKC ISO8397:2009	1 mm 98 min 0.25 mm 80 min	1 mm 98 min 0.25 mm 80 min	%
*Shape	Organoleptic	powder form	powder form	/
*Color	Organoleptic	white	white	/
*Smell	Organoleptic	no	no	/
*Solubility in water	Organoleptic	Very poorly soluble	poorly soluble	%
pH	MKC ISO13037:2011	10.78	/	/
*$CaCO_3$	Volumetric ISO10693	83.31	/	%
* Total CaO	Rulebook on inorganic fertilizers of R.M. no. 96 from 31.06.2009	35.80	35±3	%
* Total MgO	Rulebook on inorganic fertilizers of R.M. no. 96 from 31.06.2009	5.08	5±1.25	%


5


6


7

Figure 5 – 7. Mine excavation, different structure, innovative formula - Terra powder

RESULTS AND DISCUSSION

The presented table 2 shows the soil pH test during application to the vines near the soil in the area where the seedling was once grafted. Before the application, the soil condition was tested in terms of physical and chemical properties, i.e. the presence of the necessary elements in it. The table shows that in all varieties treated with the preparation - T, the pH value increased compared to those varieties that were not treated - U.

This corrects the acidity of the soil, which leads to better use of water and nutrients, resulting in stronger and healthier vines. The table also shows the results of scoring the resistance of the varieties to Botrytis and Oidium (Inglezakis, 2005). From this, it can be seen that in all varieties that were treated, an increase in the score occurs, i.e. the resistance to diseases increases, only in the Chardonnay variety this is insignificant, most likely due to the action of other factors (*Pirsaheb et al.,* 2021). The presented table 3 shows the results of measuring the removed mass of mature shoots after regular pruning of the vines. It is a parameter that shows how much growth a vine has in a year. Also, this parameter shows how much there was an increase in green mass, wood mass and fertility in the previous year and should be removed by pruning in the current year. The higher this parameter is, the stronger and more productive the vines may be. The table shows that the application of the preparation in all varieties increased the growth of the treated vines compared to those vines that were not treated and that represented the control (Inagaki *et al.,* 1996).

This effective action of the zeolite shows that it is of good quality, that is, that it is naturally free and is not saturated with a large amount of certain harmful substances that it has the ability to bind to itself and remove them from the soil or the organism (plant). The more harmful elements the zeolite has bound to itself, the lower its quality (occupied). Quality zeolite provides favorable regulation of water, air and nutrients (Chatterjee *et al.,* 2023).

The mass of removed shoots is also shown visibly in graph 1. Figures 8 to 14 show the varieties and the positive impact of the preparation on inflorescences, leaf mass, green and ripe berries in the bunch, and above all the strength and straightness of the shoots.

Table 2. Correcting soil pH and disease resistance (score)

Varieties	Correcting soil pH		Disease resistance (score)	
	U	T	U	T
Black Valandovo drenok	6.3	7.1	2	3
M. Italia	5.7	6.8	1	2
Chardonnay	5.2	6.3	2	3
Pinot noir	5.5	6.5	1	2

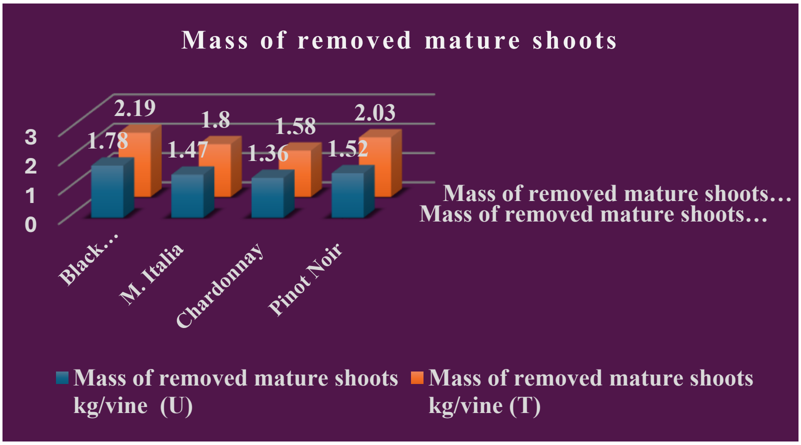
***T = Treated, *U = Untreated**

Scoring with a number: 1-weak, 2-average, 3-good, 4-very good

Table 3. Mass of removed mature shoots kg/vine

Varieties	**Mass of removed mature shoots kg/vine (U)**	**Mass of removed mature shoots kg/vine (T)**
Black Valandovo drenok	1.780	2.190
M. Italia	1.470	1.800
Chardonnay	1.360	1.580
Pinot noir	1.520	2.030
Σ	6.130	7.600
$\bar{h}$	**1.533**	**1.900**
s $\bar{h}$	0.089	0.134
σ	0.178	0.267
CV%	11.616	14.037
Xmin-	1.360	1.580
Xmax	1.780	2.190
VŠ	0.420	0.610

***T =Treated, *U = Untreated**



Graph 1. Graphical representation of the mass of removed mature shoots in grapevines

8 **9** **10**

Figure 8 – 10. Positive results in flowers, shoots and bunches

11 **12** **13** **14**

Figure 11 – 14. Black Valandovo drenok, M. Italia, Chardonnay, Pinot noir

CONCLUSIONS

From the previous analyses and obtained results, the following conclusions can be drawn:

- The preparation (the innovative formula) is a naturally good and high-quality mineral, with a good chemical composition, favorable physical properties and with its appropriate processing with certain finesse and correct application, it is a favorable means for improving the properties of the soil and grapevine.
- In our tests, the preparation showed an increase in the pH value and regulated the acidity of the soil.
- The preparation also had a favorable effect on increasing the number (scoring) for the resistance of the vines in the tested varieties. It was shown that the treated vines are plants with increased immunity to diseases.
- The treated vines also have a greater mass of removed mature shoots, thus having a greater average growth, greater green mass, larger, more beautiful and uniform berries and clusters. In fact, stronger vines are obtained, with good structure and habitus, with correctly placed shoots, without thickening, but also without rarely placed shoots.
- The preparation is suitable for use in organic grapevine production, because it can replace the use of many harmful chemicals (pesticides, herbicides, etc.). The use of this preparation does not have harmful consequences and pollution of the soil, water and air. Of course, excessive use is not necessary, because it can result in greater alkalization, and if the dosage is incorrect, it can "eat" some of the metals and a small imbalance of ions can occur. However, the negative effects are so minor in comparison to the positive effects that can be obtained from its use. This preparation is a transporter and eliminator of harmful substances and influences, it gives healthy and strong plants and also gives fertile and healthy soil. It is a boon for nature and food production.

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PRIMJENA NOVIH POBOLJŠANIH RJEŠENJA PREPARATA ZEOLITA U RAZLIČITIM FAZAMA PROIZVODNJE VINOVE LOZE U REPUBLICI SJEVERNOJ MAKEDONIJI

Sažetak

U periodu od 2018. do 2023. godine sproveli smo istraživanje o efektima primjene inovativne formule od minerala zeolita na različite faze proizvodnje vinove loze u Republici Sjevernoj Makedoniji.
Mineral zeolit potiče iz rudnika u Srbiji. U periodu od 2017. do 2018. godine napravljena je „nova formula“ od preparata zeolita uz preradu, usitnjen je u prah, sa

određenom veličinom čestica za laku primjenu u otopljenom stanju. Ova vrsta prerađenog zeolita je registrovana kao đubrivo i poboljšivač svojstava zemljišta i u tom periodu je dobila nagradu za inovaciju na sajmu u Rumuniji. Mi, u našoj zemlji, u kontinuitetu vršimo ispitivanja sa ovim preparatom u nekoliko usijeva i uvijek su davali pozitivne rezultate. U našem istraživanju prikazana su fizičko-hemijska svojstva ove vrste zeolita, a zatim rezultati i učinak primjene na vinovu lozu.
Osnovni cilj primjene ove „ poboljšane formule zeolita" bio je smanjenje upotrebe i nekontrolisane upotrebe pesticida u borbi protiv bolesti, štetočina, korova i sl. To se postiže na način da se primjenom zeolita, koji je ekološki prihvatljivih svojstva, zemljišta poboljšaju, biljka bolje koristi korisne tvari i vodu, zapravo dobiju se jače i otpornije biljke, rezistentne na bolesti, štetočine, korove, sušu, mraz itd. Istovremeno se smanjuje upotreba pesticida, a smanjuje se i zagađenje životne sredine.

Ključne riječ: *zeolit, poboljšana formula, poboljšivač svojstava, pesticidi, vinova loza*

GENETIC CHARACTERIZATION OF LOCAL COMMON BEAN LANDRACES FROM BOSNIA AND HERZEGOVINA AND REFERENCE CULTIVARS USING SSR MARKERS – INSIGHTS INTO DIVERSITY AND STRUCTURE

Merim Halilović[1], Arnela Okić[1], Almira Konjić[1], Silvio Šimon[2], Jasmin Grahić*[1]

Original scientific paper

Abstract

This aim of this study was to analyze the genetic diversity and structure of 39 common bean (*Phaseolus vulgaris* L.) accessions (35 landraces and four reference cultivars) maintained at the Gene Bank of the Faculty of Agriculture and Food Sciences in Sarajevo (Bosnia and Herzegovina), using 10 SSR markers. The analysis identified 77 alleles, averaging 7.7 alleles per locus. The calculated expected heterozygosity (H_E) was relatively high, with a mean of 0.719, whereas the observed heterozygosity (H_O) was low, averaging 0.033. These results reflect the self-pollinating nature of the analyzed plant species. The polymorphic information content (PIC) values indicate that the SSR markers used in this study, especially PVat007, BM143, and GATS91, are highly informative. STRUCTURE analysis identified three major genetic clusters (K = 3), supported by the ΔK method and further validated through factorial correspondence analysis (FCA). While most accessions were distinctly assigned to specific clusters, a small number exhibited admixed ancestry. Several local landraces clustered closely with reference cultivars, suggesting a complex gene flow and selection history. These findings highlight the significant genetic variability among Bosnia-Herzegovina's landraces and their potential importance for conservation and breeding efforts.

Keywords: *common bean, landraces, commercial cultivars, SSR markers, genetic diversity, population structure, Gene bank*

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is a globally cultivated grain legume, serving as a crucial source of dietary protein, minerals, and vitamins, particularly in developing and transitional economies. Its importance goes beyond just nutrition. It also includes cultural and agricultural heritage, especially in areas where traditional types of crops have been grown for many generations. Bosnia and Herzegovina (B&H) exemplifies such a region, where diverse local common bean landraces have been developed and preserved through farmer selection and adaptation to varied agroecological conditions.

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Local landraces are increasingly at risk of extinction due to genetic erosion, primarily caused by the widespread adoption of commercial high-yield cultivars and the decline of traditional farming practices in marginal areas. The conservation and genetic characterization of these landraces are crucial for preserving agrobiodiversity (Khoury *et al*., 2021).

Molecular markers, particularly simple sequence repeats (SSRs), are extensively used for genetic diversity analysis and cultivar identification due to their high polymorphism, reproducibility, and codominant nature. Numerous studies have underscored the value of SSRs in uncovering genetic variation among *P. vulgaris* accessions. For instance, in Croatia, Carović-Stanko *et al*. (2017) analyzed 300 accessions using 26 SSR markers and revealed a significant level of diversity, identifying both Andean and Mesoamerican gene pools along with intermediate hybrid forms. Similarly, in Serbia, Savić *et al*. (2021) utilized 27 SSR markers to investigate 136 genotypes, uncovering extensive polymorphism, with landraces exhibiting greater genetic diversity than commercial cultivars.

Similar observations have been made throughout the Western Balkans. Maras *et al*. (2015) analyzed 119 accessions from five countries of former Yugoslavia and identified 118 alleles across 13 SSR loci, indicating that most genetic variation exists within populations rather than between countries. Pipan and Meglič (2019) expanded this analysis to a broader European context, confirming the presence of high allelic richness and minimal geographic structuring among common bean landraces. Beyond the Balkans, SSR-based studies from Portugal, Turkey, and Central Europe highlight the significant genetic variability present in traditional bean germplasm (Özkan *et al*., 2022; Carvalho *et al*., 2020). These studies emphasize the potential of local varieties as valuable sources of traits for resistance to abiotic and biotic stresses, particularly under the pressures of climate change. In Bosnia and Herzegovina, the long-standing tradition of smallholder farming, coupled with mountainous terrain and agroecological heterogeneity, has likely contributed to the maintenance of diverse landraces across micro-regions. Historically, the exchange of seeds among rural communities and the practice of farmer-led selection have been essential in shaping the unique genetic profiles of local common bean varieties, helping to preserve traits that are adapted to specific environmental and cultural conditions.

Research on the diversity of common beans in B&H has primarily focused on morphological traits (Grahić *et al*., 2018; Grahić *et al*., 2013). More recent studies utilizing SSR markers (Bajrić *et al*., 2021) have uncovered the presence of both Andean and Mesoamerican gene pools within the B&H germplasm, indicating a complex history of introduction and selection. However, a thorough comparison between local landraces and reference cultivars using SSR markers has yet to be undertaken.

The objective of this study was to genetically characterize 35 common bean landraces from Bosnia and Herzegovina and compare them with 4 reference cultivars using 10 SSR markers. The study aimed to assess overall genetic diversity, identify unique or admixed genotypes, and explore the population structure within and between these two groups.

MATERIALS AND METHODS

The 39 common bean accessions (35 landraces from B&H and four reference cultivars) used in this study were obtained from the bean collection stored at the Gene Bank of the Faculty of Agriculture and Food in Sarajevo (Bosnia and Herzegovina). To acquire plant material for analysis, 3-5 seeds from each accession were sown in individual pots filled with substrate. Genomic DNA was isolated from the young, green leaves of common bean seedlings utilizing the peqGOLD plant DNA kit (Peqlab), in accordance with the manufacturer's recommended protocol. A total of ten primer pairs were used to amplify specific SSRs (Table 1). The PCR reactions were executed on a Veriti™ Thermal Cycler (Applied Biosystems®, Foster City, CA, USA), following the methodology described by Carović-Stanko *et al*. (2017).

Table 1. List of microsatellite markers used in this study. The table shows the marker name, sequence and their repetitive motifs

Markers	Forward and reverse PCR primer	Repetitive motifs
BM143	F: TGTAAACGACGGCCAGTATGCGGGAAATGAACAGAGGAAA R: ATGTTGGGAACTTTTAGTGTG	$(GA)_{35}$
GATS91	F: TGTAAACGACGGCCAGTATGCGAGTGCGGAAGCGAGTAGAG R: TCCGTGTTCCTCTGTCTGTG	$(GA)_{17}$
BM210	F: TGTAAACGACGGCCAGTATGCACCACTGCAATCCTCATCTTTG R: CCCTCATCCTCCATTCTTATCG	$(CT)_{15}$
BM151	F: TGTAAACGACGGCCAGTATGCCACAACAAGAAAGACCTCCT R: TTATGTATTAGACCACATTACTTCC	$(TC)_{14}$
PVag001	F: TGTAAACGACGGCCAGTATGCCAATCCTCTCTCTCTCATTCCAATC R: GACCTTGAAGTCGGTGTCGTTT	$(GA)_{11}$
BMb174	F: TTGAAACAAATCAGACCCTC R: ATACATAGATGCAAGAGCGA	$(TTA)_{10}$
PVat007	F: TGTAAACGACGGCCAGTATGCAGTTAAATTATACGAGGTTAGCCTAAATC R: CATTCCCTTCACACATTCACCG	$(AT)_{12}$
BMd42	F: TGTAAACGACGGCCAGTATGCTCATAGAAGATTTGTGGAAGCA R: TGAGACACGTACGAGGCTGTAT	$(AT)_{5}$
BMb96	F: CATAAAGCACGTCACTTCAA R: GCCTTGGACACTACCATTT	$(CA)_{11}$
BMb247	F: ATCCTAGGGAGTCATGAAGG R: AGAATTGTAACCACACCGAC	$(AT)_{13}$

PCR amplification was carried out in a total volume of 20 µL. Each reaction mixture comprised of 2 pmol of the tailed forward primer, 8 pmol of the reverse primer, 8 pmol of the FAM-labeled M13 primer, 1 × PCR buffer, 4 pmol of each dNTP, 0.5 U of TaqTM HS DNA Polymerase (Takara Bio Inc., Japan), and 5 ng of genomic DNA. Genetic diversity parameters—namely, the mean number of alleles per locus (Na),

observed heterozygosity (H_O), and expected heterozygosity (H_E) —were computed for each of the 10 SSR loci using the software SpaGedi v1.2 (Hardy and Vekemans, 2002). PIC value was calculated using Cervus v.3.0.7 (Kalinowski *et al*., 2007).
To examine the population structure, a Bayesian model-based clustering procedure was applied using Structure version 2.2.3 (Pritchard *et al*., 2000). The analysis computed K (unknown) RPPs (reconstructed panmictic populations) for individuals testing K (log-likelihood) values ranging from 1 to 10 for all accessions, assuming that the sampled cultivars originated from an unknown source. Although the dataset included known reference cultivars, all accessions were treated as having unknown origin in the STRUCTURE analysis to allow unbiased inference of genetic structure and potential admixture patterns without using prior classification. For each K, ten independent runs were executed. The tests utilized an admixture model where allelic frequencies were correlated and assumed varying Fst values for specific subpopulations, with a burn-in period of 200,000 followed by 500,000 iterations.
To determine the most likely number of genetic clusters (K), the StructureSelector tool (Li and Liu, 2018) was employed, using the ΔK method developed by Evanno *et al.* (2005). Following the estimation of the optimal K value, individuals were grouped into specific clusters (Vigouroux *et al*., 2008) based on the run with the highest likelihood. Additional cluster assignments, which were based on the average of membership probability values over the ten runs, confirmed the initial results. Cultivars were assigned to specific RPPs when their qI values reached or exceeded 95%. To explore genetic relationships among individuals, a factorial correspondence analysis (FCA) was performed using allele frequency data in Genetix (Belkhir *et al*., 2004). All datasets used for statistical procedures were formatted with MADC v2.0 software package (Grahić and Grahić, 2017).

RESULTS AND DISCUSSION

A total of 10 SSR markers were utilized to genotype 39 common bean accessions, which included 35 local landraces from B&H and 4 reference cultivars. The number of alleles per locus (Na) varied from 4 (for PVag001 and BMb247) to 13 (for GATS91 and PVat007), resulting in an overall mean of 7.7 alleles per locus (Table 2). Notably, the highest levels of polymorphism were recorded for PVat007 and GATS91, underscoring their marked informativeness, which is consistent with previous studies on Balkan and European bean germplasms (Pipan and Meglič, 2019; Carović-Stanko *et al*., 2017; Maras *et al*., 2015).

Table 2. Characterization of the 10 microsatellite loci used on 39 common bean accessions (35 local varieties and 4 reference cultivars)

Analyzed loci	Na	Range (bp)	H_O	H_E	PIC
BM143	11.0	110/182	0.051	0.835	0.803
GATS91	13.0	212/258	0.077	0.814	0.787
BM210	6.0	158/184	0.000	0.718	0.665
BM151	7.0	140/163	0.051	0.675	0.615
PVag001	4.0	150/160	0.000	0.690	0.620
BMb174	6.0	87/158	0.000	0.593	0.553
PVat007	13.0	192/226	0.077	0.865	0.841
BMd42	8.0	103/161	0.051	0.683	0.629
Bmb96	5.0	118/126	0.000	0.721	0.667
Bmb247	4.0	131/139	0.027	0.599	0.540
Mean	**7.7**		**0.033**	**0.719**	**0.672**

Na – number of alleles; H_O - observed heterozygosity; H_E - expected heterozygosity; PIC - polymorphic information content

Observed heterozygosity (H_O) values were generally low, with a mean of 0.033, reflecting the primarily self-pollinating nature of *Phaseolus vulgaris* L., as noted in other studies (Blair *et al*., 2006; Beebe *et al*., 2001). In contrast, expected heterozygosity (H_E) was significantly higher, with a mean of 0.719, highlighting a rich allelic diversity within the studied germplasm, which aligns well with previous research on European landraces based on both molecular and biochemical markers (De la Fuente *et al*., 2012; Angioi *et al*., 2010). Polymorphic information content (PIC) values spanned from 0.540 to 0.841, with an average value of 0.672, indicating that most of the markers used were highly informative (PIC > 0.5), particularly PVat007 (0.841), BM143 (0.803), and GATS91 (0.787). These findings are consistent with those reported by Bajrić *et al*. (2021) for B&H germplasm, while similar results have also been documented in studies from Portugal (Carvalho *et al*., 2020) and Serbia (Savić *et al*., 2021). These results underscore the effectiveness of the selected SSR markers in capturing genetic variation and support their ongoing use in assessments of common bean diversity, particularly in studies involving a mix of local and commercial germplasm (Blair *et al*., 2009).

The Bayesian clustering analysis conducted using STRUCTURE software identified three distinct genetic clusters (K = 3), as determined by the ΔK method (Evanno *et al*., 2005) and further validated via StructureSelector (Li and Liu, 2018) (Figure 1).

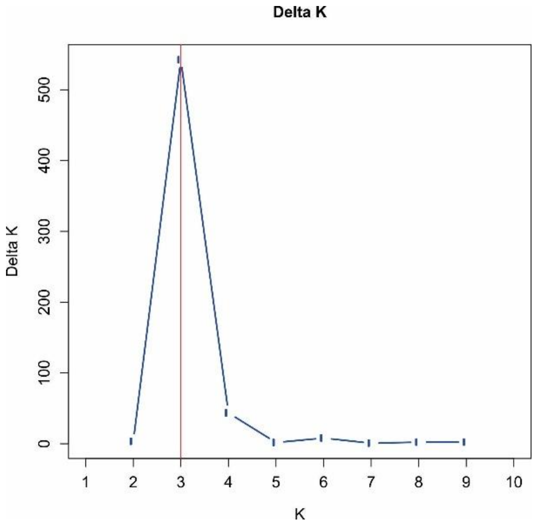

Figure 1. Plot of ΔK values (Evanno *et al*., 2005) from the Structure analyses of 39 common bean accessions obtained through StructureSelector (Li and Liu, 2018)

The bar plot illustrating individual membership probabilities (Figure 2.) clearly demonstrated that most accessions were assigned to one of the three reconstructed panmictic populations (RPPs), although several accessions exhibited admixed genetic profiles.

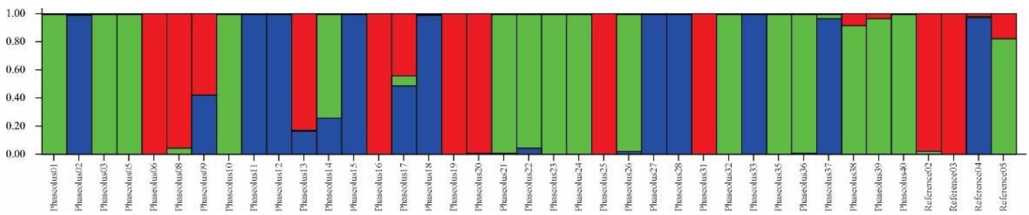

Figure 2. Bar plot of the results from three Bayesian genetic structure analyses of 39 common bean accessions with K = 3. The bar plot clearly indicates the existence of three panmictic populations (RPPs). Bayesian bar plot was generated using STRUCTURE software

This pattern of genetic structuring closely resembles findings reported for European and Balkan common bean germplasm (Maras *et al*., 2015; Bitocchi *et al*., 2012). Out of 39 accessions, 33 were confidently assigned to one of the three RPPs (qI > 95%), while 6 accessions displayed evidence of admixture (Table 3). Reference cultivars were allocated to RPPs 1 and 3, whereas local landraces were distributed across all three

RPPs, indicating a rich and complex genetic background within the local materials. Notably, landraces clustered alongside reference cultivars suggest possible historical gene flow or shared ancestry between commercial and traditional varieties (Blair *et al*., 2006; Papa and Gepts, 2003).

Table 3. Analyzed common bean samples assigned to a reconstructed panmictic population (RPP), for K = 3, with probability of membership qI > 95%.

Sample	**RPP, K = 3**	**Sample**	**RPP, K = 3**	**Sample**	**RPP, K = 3**
Phaseolus01	2	Phaseolus16	1	Phaseolus31	1
Phaseolus02	3	Phaseolus17	Admixed	Phaseolus32	2
Phaseolus03	2	Phaseolus18	3	Phaseolus33	3
Phaseolus05	2	Phaseolus19	1	Phaseolus35	2
Phaseolus06	1	Phaseolus20	1	Phaseolus36	2
Phaseolus08	1	Phaseolus21	2	Phaseolus37	3
Phaseolus09	Admixed	Phaseolus22	2	Phaseolus38	Admixed
Phaseolus10	2	Phaseolus23	2	Phaseolus39	2
Phaseolus11	3	Phaseolus24	2	Phaseolus40	2
Phaseolus12	3	Phaseolus25	1	Reference02	1
Phaseolus13	Admixed	Phaseolus26	2	Reference03	1
Phaseolus14	Admixed	Phaseolus27	3	Reference04	3
Phaseolus15	3	Phaseolus28	3	Reference05	Admixed

Factorial correspondence analysis (FCA) was initially conducted on all 39 accessions, encompassing both local landraces and reference cultivars. This preliminary analysis uncovered three primary spatial groupings, with several local landraces situated in close proximity to reference cultivars (Phaseolus06, Phaseolus08, and Phaseolus25), as shown in Figure 3. Such positioning implies potential genetic similarity between specific traditional and commercial genotypes, a phenomenon previously noted in comparable studies (Angioi *et al*., 2010; Blair *et al*., 2009).

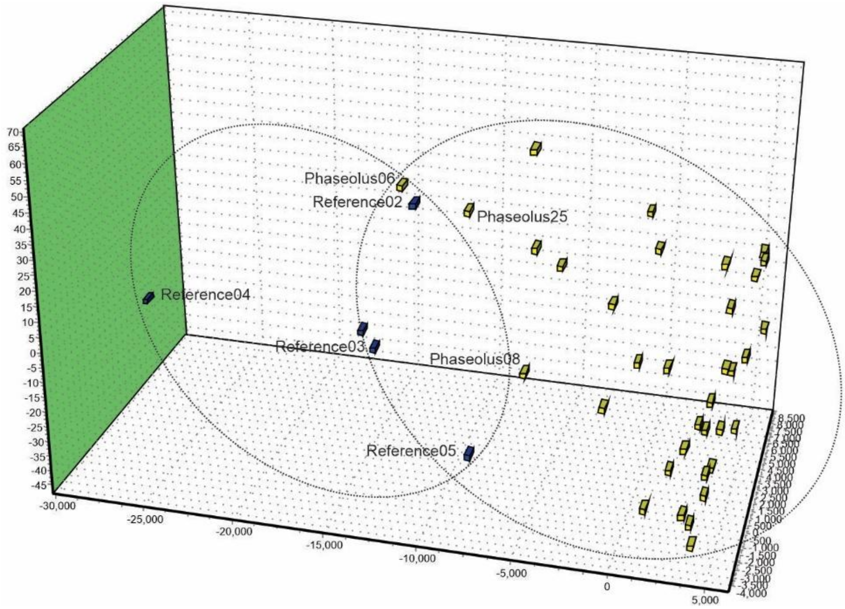

Figure 3. Multivariate analysis (FCA) of SSR data for 39 common bean accessions (35 landraces and 4 reference cultivars) calculated using Genetix

Subsequent to the STRUCTURE analysis, which classified accessions into three reconstructed panmictic populations (RPPs), a second FCA was performed using only those accessions with clear membership coefficients (qI > 95%). In this refined analysis, admixed individuals were excluded to enhance the resolution of population-level structure. The resulting FCA plot corroborated the clustering inferred by STRUCTURE, with each RPP forming a distinct spatial group that displayed internal coherence (Figure 4.). The alignment between the results of STRUCTURE and FCA underlines the reliability of the identified population structure and emphasizes the presence of distinct genetic backgrounds among the studied accessions. The close positioning of certain local landraces to reference cultivars in the initial FCA hints at a complex history of introduction, selection, or gene flow within the B&H germplasm, similar to findings reported in studies from the Western Balkans and Southern Europe (Pipan and Meglič, 2019; Angioi *et al.,* 2010).

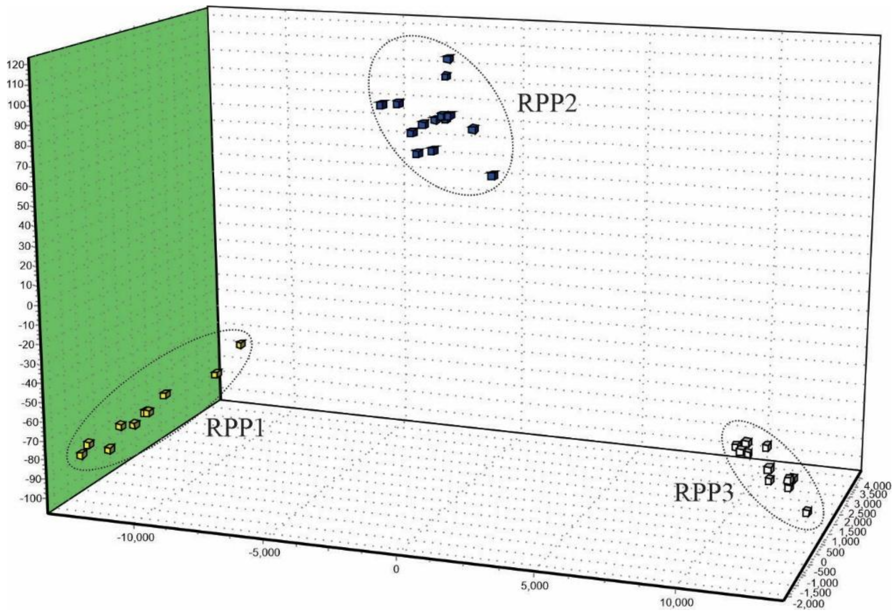

Figure 4. Multivariate analysis (FCA) of SSR data for three defined reconstructed panmictic populations (RPP) calculated using STRUCTURE (Pritchard et al., 2000) (only genotypes with likelihood of membership to individual RPP above 95% are included in the analyses)

CONCLUSIONS

The results of this study confirm significant genetic diversity among the local common bean landraces from Bosnia and Herzegovina. These landraces demonstrate both genetic uniqueness and some overlap with reference cultivars. Identifying three principal genetic groups, supported by STRUCTURE and FCA analyses and the presence of admixed genotypes, highlights the complexity and richness of the local germplasm. Understanding this structure enables the identification of genetically unique accessions that should be prioritized for ex-situ conservation. At the same time, admixed genotypes may represent valuable sources of genetic recombination and evolutionary adaptation. Incorporating both distinct and admixed materials ensures that gene banks capture the full spectrum of diversity, supporting long-term preservation and breeding resilience.
These results highlight the importance of conserving local landraces as invaluable genetic resources. Their diversity forms a critical foundation for future breeding programs to enhance stress resilience, yield stability, and adaptability to local conditions. In addition, the informative SSR markers discovered in this research,

particularly PVat007, BM143, and GATS91, demonstrate strong potential for diversity evaluations, core collection creation, and strategies for marker-assisted selection.

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GENETIČKA KARAKTERIZACIJA LOKALNIH SORTI OBIČNOG GRAHA IZ BOSNE I HERCEGOVINE I REFERENTNIH KULTIVARA POMOĆU SSR MARKERA – UVID U GENETIČKU RAZNOLIKOST I STRUKTURU

Sažetak

Cilj ove studije bio je analizirati genetičku raznolikost i strukturu 39 primki običnog graha (*Phaseolus vulgaris* L.), odnosno 35 lokalnih sorti i četiri referentna kultivara, koji se čuvaju u Gen banci Poljoprivredno-prehrambenog fakulteta u Sarajevu, korištenjem 10 SSR markera. Analizom je identificirano ukupno 77 alela, s prosjekom od 7,7 alela po lokusu. Očekivana heterozigotnost (H_E) bila je relativno visoka (prosječna vrijednost za sve lokuse je 0,719), dok je ustanovljena heterozigotnost (H_O) bila niska (prosječno 0,033), što odražava samooplodnu prirodu analizirane biljne vrste. PIC vrijednosti ukazuju da su korišteni SSR markeri, posebno PVat007, BM143 i GATS91, visoko informativni. Analizom populacijske strukture (STRUCTURE) identifikovane su tri glavne genetičke grupe (K = 3), što je dodatno validirano faktorskom analizom korespondencije (FCA). Većina primki je jasno svrstana u specifične klastere, dok je mali broj pokazao mješovito genetičko porijeklo. Nekoliko lokalnih sorti grupisano je blizu referentnih kultivara, što ukazuje na kompleksnu historiju protoka gena i selekcije. Dobijeni rezultati potvrđuju visoku genetičku raznolikost među lokalnim kultivarima graha iz Bosne i Hercegovine i ukazuju na važnost njihovog očuvanje za buduče oplemenjivačke procese.

Ključne riječi: *obični grah, lokalne sorte, kultivari, SSR markeri, genetički diverzitet, populacijska struktura, Gen banka*

MARKER-ASSISTED SCREENING FOR POTENTIAL DUPLICATES AMONG COMMON BEAN GENE BANK COLLECTION

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Original scientific paper

Abstract

The characterization and preservation of local plant genetic resources are crucial for maintaining agrobiodiversity and enhancing crop improvement initiatives. This study aimed to evaluate the genetic diversity and identify potential duplicates among 21 local common bean (*Phaseolus vulgaris* L.) accessions stored in the Gene Bank of the Faculty of Agriculture and Food Sciences at the University of Sarajevo. A total of 26 SSR (single sequence repeat) markers were utilized to genotype the accessions, revealing an average of 4.0 alleles per locus. The observed heterozygosity (H_O) averaged 0.043, while the expected heterozygosity (H_E) was 0.536. The polymorphic information content (PIC) values ranged from 0.171 to 0.859, with 11 loci classified as highly informative (PIC > 0.5). Cluster analysis based on Jaccard's distance and the UPGMA method indicated that no two accessions shared identical allele profiles, confirming the absence of genetic duplicates within the analyzed group. These findings highlight the significance of conserving and further characterizing local common bean germplasm as a vital resource for breeding programs focused on improving yield, stress resilience, and adaptation. Additionally, several SSR loci, especially PVat007, BM143, and GATS91, were identified as highly informative and suitable for future diversity and selection studies.

Keywords: *common bean, SSR markers, genetic diversity, Gene bank, landraces, duplicate identification*

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is a crucial food legume globally, serving as a key source of dietary protein, minerals, and vitamins, particularly in traditional farming systems. In Bosnia and Herzegovina (B&H), the common bean is cultivated across a range of agroecological zones, where farmers have historically preserved diverse landraces that are well-adapted to local environmental conditions and cultural practices. These traditional varieties represent a valuable reservoir of genetic diversity,

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which is vital for food security, especially in the context of climate change and resource-limited agriculture (Grahić *et al*., 2013; Grahić *et al*., 2018). According to the 2023 report "Harvested area and yield of major crops", issued by the Agency for Statistics of Bosnia and Herzegovina, the dry common bean was harvested from 2,782 hectares, with an average yield of 3.51 t/ha (Agency for Statistics of B&H, 2023). These figures underscore the continued importance of this crop within smallholder and traditional farming systems across the country. Despite its significance, the genetic diversity of common bean landraces in B&H has not been extensively characterized using molecular markers. Previous studies have mainly focused on morphological traits (Grahić *et al*., 2013) and their effectiveness in determining the origins of gene pools (Grahić *et al*., 2018). While these methods provide valuable insights, their resolution is limited. Microsatellite markers (SSRs) are recognized for their efficacy in characterizing genetic diversity and remain an important tool for identifying distinct and potentially duplicated accessions (Carović-Stanko *et al*., 2017; Savić *et al*., 2021).
SSR markers are highly effective for characterizing common bean germplasm due to their considerable polymorphism, reproducibility, and codominant inheritance. They have been extensively employed in the Balkans and Europe to differentiate between landraces and commercial varieties of different agricultural crops, detect admixed genotypes, and inform conservation strategies (Maras *et al*., 2015; Carović-Stanko *et al.,* 2017; Pipan and Meglič, 2019). A recent study focusing on B&H common bean germplasm, utilizing SSR analysis, confirmed the presence of both Andean and Mesoamerican gene pools within the national collection, shedding light on historical hybridization events and significant intraspecific diversity (Bajrić *et al.,* 2021). Given the increasing threat of genetic erosion due to the replacement of traditional varieties with commercial cultivars, it is essential to characterize and conserve local landraces. The Gene Bank at the Faculty of Agriculture and Food Sciences, University of Sarajevo, has successfully gathered and preserved a substantial number of common bean accessions from across Bosnia and Herzegovina. However, there remains uncertainty regarding the extent to which these accessions represent genetically distinct materials and the potential existence of duplicates.
This study employs 26 SSR markers to assess genetic diversity and identify possible duplicates among 21 local common bean accessions held in the Sarajevo Gene Bank. The results will enhance the efficient conservation, management, and utilization of local common bean genetic resources for breeding programs, thereby promoting sustainable agricultural development.

MATERIALS AND METHODS

For molecular analysis, 3 to 5 seeds from each of the 21 common bean (*Phaseolus vulgaris* L.) accessions stored in the Gene Bank of the Faculty of Agriculture and Food Sciences University of Sarajevo were individually sown in pots filled with commercial substrate. Genomic DNA was extracted from the young green leaves of the resulting seedlings using the peqGOLD Plant DNA Kit (Peqlab, Germany), following the manufacturer's instructions.

A total of 26 primer pairs were utilized for SSR marker amplification (as detailed in Table 1). The PCR reactions were conducted using a Veriti™ Thermal Cycler (Applied Biosystems®, Foster City, California, USA), according to the protocol described by Carović-Stanko *et al*. (2017). Each 20 µL PCR reaction mixture contained 2 pmol of a tailed forward primer, 8 pmol of a reverse primer, 8 pmol of a FAM-labeled M13 primer, 1× PCR buffer, 4 pmol of each dNTP, 0.5 U of Taq™ HS DNA Polymerase (Takara Bio Inc.), and 5 ng of genomic DNA.

Table 1. Microsatellite markers used in this study The table shows the marker name, sequence, and their repetitive motifs.

Markers	**Forward and reverse PCR primer**	**Repetitive motifs**
BM157	F: ACTTAACAAGGAATAGCCACACA R: GTTAATTGTTTCCAATATCAACCTG	$(GA)_{16}$
BM172	F: TGTAAACGACGGCCAGTATGCCTGTAGCTCAAACAGGGCACT R: GCAATACCGCCATGAGAGAT	$(GA)_{23}$
BM143	F: TGTAAACGACGGCCAGTATGCGGGAAATGAACAGAGGAAA R: ATGTTGGGAACTTTTAGTGTG	$(GA)_{35}$
GATS91	F: TGTAAACGACGGCCAGTATGCGAGTGCGGAAGCGAGTAGAG R: TCCGTGTTCCTCTGTCTGTG	$(GA)_{17}$
Pvctt001	F: TGTAAACGACGGCCAGTATGCGAGGGTGTTCACTATTGTCACTGC R: TTCATGGATGGTGGAGGAACAG	$(CTT)_3(T)_3(CTT)_6$
BM210	F: TGTAAACGACGGCCAGTATGCACCACTGCAATCCTCATCTTTG R: CCCTCATCCTCCATTCTTATCG	$(CT)_{15}$
BMd20	F: TGTAAACGACGGCCAGTATGCGTTGCCACCGGTGATAATCT R: GTGAGGCAAGAAGCCTTCAA	$(TA)_5$
BMd12	F: TGTAAACGACGGCCAGTATGCCATCAACAAGGACAGCCTCA R: GCAGCTGGCGGGTAAAACAG	$(AGC)_7$
BM151	F: TGTAAACGACGGCCAGTATGCCACAACAAGAAAGACCTCCT R: TTATGTATTAGACCACATTACTTCC	$(TC)_{14}$
PVag001	F: TGTAAACGACGGCCAGTATGCCAATCCTCTCTCTCTCATTCCAATC R: GACCTTGAAGTCGGTGTCGTTT	$(GA)_{11}$
PVat007	F: TGTAAACGACGGCCAGTATGCAGTTAAATTATACGAGGTTAGCCTAAATC R: CATTCCCTTCACACATTCACCG	$(AT)_{12}$
BMd42	F: TGTAAACGACGGCCAGTATGCTCATAGAAGATTTGTGGAAGCA R: TGAGACACGTACGAGGCTGTAT	$(AT)_5$
BMd45	F: TGTAAACGACGGCCAGTATGCGGTTGGGAAGCCTCATACAG R: ATCTTCGACCCACCTTGCT	$(AG)_5$
BMb247	F: ATCCTAGGGAGTCATGAAGG R: AGAATTGTAACCACACCGAC	$(AT)_{13}$
BMb96	F: CATAAAGCACGTCACTTCAA R: GCCTTGGACACTACCATTT	$(CA)_{11}$
BMd22	F: GGTCACTTCCGGAGCATTC R: CGGGAAATGGAAGTCACAGT	$(TC)_6$
BMb508	F: TTGAGACAAATGACTCACCA R: CGTGTTCCTTTAAACAATCC	$(CA)_{11}$
BMd47	F: TGTAAACGACGGCCAGTATGCACCTGGTCCCTCAAACCAAT R: CAATGGAGCACCAAAGATCA	$(AT)_5$
BMb469	F: CATTCATGTGAACCTTTCATT R: ATTGTTTGGTTTGTGCTTCT	$(AGA)_{10}$
BMd46	F: GGCTGACAACAACTCTGCAC R: CTGGCATAGGTTGCTCCTTC	$(TCT)_4$
BM197	F: TGGACTGGTCGATACGAAGC R: CCCAGAAGATTGAGAACACCAC	$(GT)_8$
BMb267	F: TGAGCATCCTCTACTTGGTT R: AATCTCGCCTCTCTCTCTTT	$(GAA)_9$

BMd53	F: TGTAAACGACGGCCAGTATGCTGCTGACCAAGGAAATTCAG	$(GTA)_5$
	R: GGAGGAGGCTTAAGCACAAA	
PVag003	F: TCACGTACGAGTTGAATCTCAGGAT	$(AG)_8$
	R: GGTGTCGGAGAGGTTAAGGTTG	
BMb174	F: TTGAAACAAATCAGACCCTC	$(TTA)_{10}$
	R: ATACATAGATGCAAGAGCGA	
BMd25	F: TGTAAACGACGGCCAGTATGCGCAGATCGCCTACTCACAAA	$(GAT)_6$
	R: CGTTGACGAGAAGCATCAAG	

To calculate genetic diversity parameters, including the number of alleles per locus (Na), observed heterozygosity (H_O), and expected heterozygosity (H_E), SpaGeDi v.1.2 software was employed (Hardy and Vekemans, 2002). The polymorphic information content (PIC) was calculated using Cervus v.3.0.7 (Kalinowski *et al*., 2007).
Genetic distance among accessions was visualized using the Jaccard distance and clustered by the UPGMA method (Unweighted Pair Group Method with Arithmetic Mean) in the MEGA 6 software (Tamura *et al*., 2013). All input data for statistical analysis were prepared using MADC v.2.0 software (Grahić and Grahić, 2017).

RESULTS AND DISCUSSION

A total of 26 microsatellite loci were successfully amplified from 21 local common bean accessions collected from various regions of Bosnia and Herzegovina (B&H), covering a wide range of agroecological zones, including continental lowlands, hilly regions, and mountainous rural areas. This geographic diversity ensures that the genetic variation assessed in this study accurately reflects the traditional cultivation range of common beans within the country. The SSR markers demonstrated significant genetic variability among the accessions, highlighting the effectiveness of SSRs for studying diversity in *Phaseolus vulgaris*.

Table 2. Characterization of the 26 microsatellite loci used on 21 common bean accessions

Analyzed loci	Na	Range (bp)	H_O	H_E	PIC
BM157	4.0	96/114	0.048	0.603	0.546
BM172	4.0	81/109	0.000	0.562	0.495
BM143	7.0	112/152	0.000	0.832	0.790
GATS91	9.0	216/258	0.143	0.816	0.778
Pvctt001	4.0	153/163	0.095	0.548	0.436
BM210	5.0	162/188	0.048	0.710	0.645
BMd20	2.0	116/120	0.000	0.316	0.261
BMd12	3.0	151/163	0.048	0.554	0.465
BM151	4.0	139/149	0.000	0.660	0.581
PVag001	5.0	150/160	0.048	0.717	0.650
PVat007	11.0	192/224	0.095	0.893	0.859
BMd42	6.0	105/161	0.095	0.683	0.632
BMd45	2.0	88/126	0.048	0.494	0.366

BMb247	4.0	131/139	0.053	0.622	0.548
BMb96	5.0	114/197	0.048	0.758	0.699
BMd22	2.0	116/118	0.048	0.470	0.354
BMb508	3.0	244/250	0.050	0.432	0.365
BMd47	3.0	150/154	0.000	0.455	0.398
BMb469	2.0	141/147	0.053	0.235	0.202
BMd46	3.0	322/328	0.000	0.400	0.355
BM197	2.0	200/202	0.095	0.251	0.215
BMb267	2.0	223/226	0.000	0.193	0.171
BMd53	2.0	103/106	0.048	0.494	0.366
PVag003	2.0	163/165	0.000	0.455	0.346
BMb174	5.0	89/197	0.053	0.292	0.274
BMd25	2.0	115/118	0.000	0.483	0.360
Mean	**4.0**		**0.043**	**0.536**	**0.468**

Na - number of alleles; H_O - observed heterozygosity; H_E - expected heterozygosity; PIC - polymorphic information content.

The number of alleles per locus (Na) ranged from 2 to 11, with an average of 4.0 alleles per locus. The most polymorphic loci included PVat007 (Na = 11), GATS91 (Na = 9), and BM143 (Na = 7), which aligns with their previously reported informativeness in common bean research (Carvalho *et al*., 2020; Gioia *et al*., 2019; Carović-Stanko *et al*., 2017) (Table 2.). Observed heterozygosity (H_O) was low (mean = 0.043), which is typical for a predominantly self-pollinating species like the common bean. In contrast, the expected heterozygosity (H_E) was higher (mean = 0.536), reflecting the underlying allelic diversity present within the collection. Polymorphic information content (PIC) values varied significantly, ranging from 0.171 (BMb267) to 0.859 (PVat007), with an average of 0.468. According to Botstein's classification, markers with PIC values greater than 0.5 are deemed highly informative. In this study, 11 loci exceeded this threshold, indicating that nearly half of the SSR markers utilized are suitable for diversity and differentiation analyses within local common bean germplasm. These findings are consistent with previous SSR-based studies on common bean landraces in the Western Balkans (Maras *et al*., 2015; Savić *et al*., 2021) and align with recent research on Bosnian accessions, where similar PIC values and allele ranges have been reported (Bajrić *et al*., 2021). The presence of multiple alleles per locus reaffirms that the gene bank collection conserves a broad genetic base, highlighting its importance for conservation and breeding efforts.

The UPGMA dendrogram, which is based on Jaccard's genetic distance, categorized the 21 accessions into several clusters. Notably, no two accessions displayed identical allele profiles (Figure 1.). This finding strongly indicates the absence of genetic duplicates within the analyzed set, affirming the authenticity and uniqueness of each accession. Although a few accessions, such as BH04 and BH09, as well as BH11 and BH12, formed closely related pairs or subclusters, their profiles still exhibited sufficient allelic differences to classify them as genetically distinct. This clustering pattern aligns with the observations of Grahić *et al*. (2013), who noted that landraces demonstrated gradual genetic differentiation rather than strict organization based on geographic origin. This pattern may reflect historical seed exchange practices among farming communities within B&H, where traditional landraces were frequently shared between regions regardless of geographic proximity. Such informal exchange systems could explain the presence of genetically similar but geographically dispersed accessions, contributing to a mosaic-like distribution of genetic variation. While specific accessions were found to be more closely related than others, no highly uniform subgroups emerged. Similar patterns of diversity have been documented in other studies focusing on landraces, wherein clustering reflects intricate genetic backgrounds rather than clear-cut substructuring (Maras *et al*., 2015).

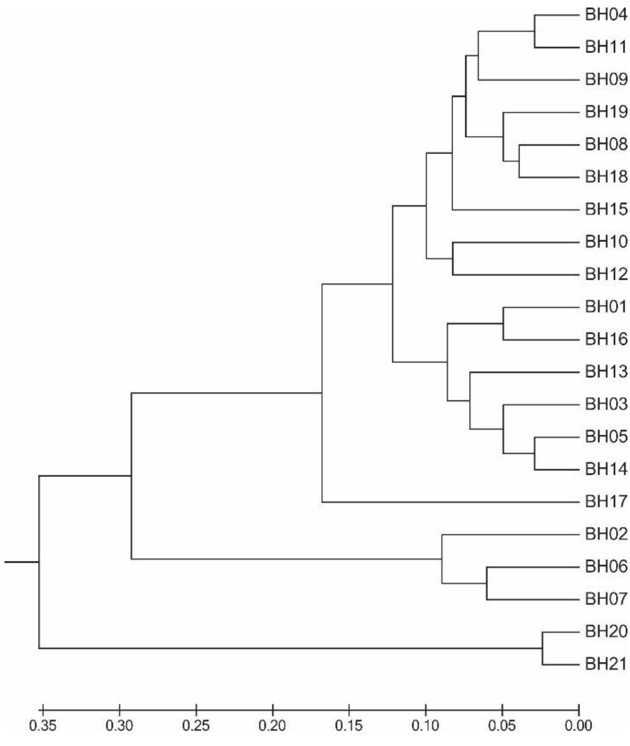

Figure 1. UPGMA cluster analysis based on polymorphisms of 26 SSR loci in 21 common bean accessions using Jaccards distance generated in MEGA 6 software (Tamura *et al*., 2013). The findings indicate the absence of duplicates

CONCLUSIONS

The results of this study confirm the significant genetic diversity present among local common bean (*Phaseolus vulgaris* L.) accessions stored in the Gene Bank of the Faculty of Agriculture and Food Sciences in Sarajevo. No genetically redundant accessions were identified within the analyzed subset, indicating that each represents a unique genotype. These findings underscore the critical importance of preserving and further characterizing this germplasm, which serves as a valuable genetic resource for future breeding efforts aimed at improving stress resilience, yield, and adaptability.

In addition to molecular characterization, further evaluation of these accessions should include detailed phenotypic assessments, particularly for traits related to drought tolerance, disease resistance, and agronomic performance under low-input conditions. Such integrated characterization would enhance their practical value and facilitate their inclusion in targeted breeding programs.

Molecular tools remain essential forprioritizing unique accessions for conservation. Furthermore, the highly informative SSR loci identified in this study, especially PVat007, BM143, and GATS91, may prove effective tools for future germplasm screening and marker-assisted selection programs.

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MARKERIMA POTPOMOGNUTI SKRINING POTENCIJALNIH GENETIČKIH DUPLIKATA U KOLEKCIJI OBIČNOG GRAHA POHRANJENOJ U GEN-BANCI

Sažetak

Detaljna genetička karakterizacija lokalnih sorti graha od ključne je važnosti za njihovo očuvanje i buduću upotrebu u oplemenjivanju i selekciji. Ova studija imala je za cilj procijeniti genetičku raznolikost i identificirati moguće duplikate između 21 lokalne primke običnog graha (*Phaseolus vulgaris* L.) pohranjene u gen banci Poljoprivredno-prehrambenog fakulteta Univerziteta u Sarajevu. Za genotipizaciju je korišteno ukupno 26 SSR markera, pri čemu je prosječan broj alela po lokusu iznosio 4,0. Ustanovljena heterozigotnost (H_O) iznosila je, u prosjeku, 0,043, dok je prosječna očekivana heterozigotnost (H_E) imala vrijednost od 0,536. Vrijednosti polimorfnog informativnog sadržaja (PIC) kretale su se od 0,171 do 0,859, pri čemu je 11 lokusa klasificirano kao visoko informativno (PIC > 0,5). Klaster analiza zasnovana na Jaccardovoj genetičkoj distanci i UPGMA metodi pokazala je da nijedna od analiziranih primki ne dijeli identičan alelni profil, što potvrđuje odsustvo genetičkih duplikata u okviru ispitivane kolekcije. Dobijeni rezultati ističu značaj očuvanja i daljnje karakterizacije lokalnog sortimenta graha kao vrijednog genetičkog resursa za oplemenjivačke programe usmjerene na poboljšanje prinosa, otpornosti na stres i adaptabilnosti. Također, nekoliko SSR lokusa, naročito PVat007, BM143 i GATS91, izdvojilo se kao visoko informativno i pogodno za buduće studije diverziteta i selekcije.

Ključne riječi: *obični grah, SSR markeri, genetički diverzitet, Gen banka, lokalne sorte, identifikacija duplikata*

SNP2STR: A USER FRIENDLY SOFTWARE FOR CREATING INPUT FILES FOR STRUCTURE SOFTWARE FROM SNP DATA*

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Original scientific paper

Abstract

STRUCTURE is a software package that uses molecular data from multiple loci to analyze population structure. To analyze Single Nucleotide Polymorphism (SNP) data using STRUCTURE, a specific input file format is required. However, because SNP studies often involve thousands of loci, these input files are typically too large to edit manually.
To address this challenge, several software tools and functions have been developed. However, in the case of plant data, some of these tools either fail to process the data correctly or generate inaccurate input files.
To overcome these issues, a new software tool - SNP2STR - has been developed. SNP2STR converts SNP data into the input format required by Structure. It generates a file with an optional first row containing marker names, followed by two rows of data per individual, formatted according to the corresponding markers.
The software is open-source and available on GitHub. It also offers potential for future upgrades based on user feedback.

Key words: *molecular data, population structure analysis, genetic clustering, Open-source bioinformatics tools*

INTRODUCTION

In recent decades, plant genetic analysis has undergone a significant transformation by applying molecular markers, catalyzing advancements in molecular breeding in agriculture (Mammadov *et al*., 2012). Early-generation markers, such as restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPDs), and amplified fragment length polymorphisms (AFLPs), were instrumental in initial genetic studies but were limited by low throughput, poor reproducibility, or complex protocols (Varshney *et al*., 2007; Mammadov *et al*., 2012; Mueller *et al*., 1999). These were followed by second-generation markers like simple sequence repeats (SSRs or microsatellites), which offered improved reproducibility and informativeness

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(Powell *et al*., 1996). More recently Single nucleotide polymorphisms (SNPs) have become the preferred choice among these markers due to their abundance, genome-wide distribution, and suitability for high-throughput genotyping. SNP markers enable detailed investigations into genetic diversity, linkage mapping, and marker-assisted selection in various plant species (Ganal *et al*., 2009). The availability of high-density SNP datasets has also facilitated powerful approaches such as whole-genome scanning (WGS), genome-wide association studies (GWAS), and association genetics, allowing researchers to link genomic variation to phenotypic traits at unprecedented resolution (Zhu *et al*., 2008).

As SNP genotyping technologies now routinely generate hundreds of thousands of markers, with densities reaching up to 480K per individual, the resulting datasets are large and complex, requiring efficient computational tools for downstream analysis. Several programs have been developed for handling and analyzing such data, including PLINK (Purcell *et al*., 2007), VCF2PopTree (Subramanian *at al*., 2019), and R-based tools (R Core Team, 2024). However, population structure analysis, an essential step in assessing genetic subgroups and potential admixture, often relies on programs such as STRUCTURE (Pritchard *et al*., 2000) and fastSTRUCTURE (Raj *et al.,* 2014). These tools use multi-locus genotype data to infer hidden population structure, estimate ancestry proportions, and assign individuals to genetic clusters (Pritchard *et al*., 2000, Raj *et al*., 2014, Falush *et al*., 2003). This is especially important in studies of plant genetics focused on managing genetic resources, enhancing breeding strategies, and detecting gene flow between cultivars and their wild relatives.

The STRUCTURE software implements a Bayesian model-based clustering method that infers population structure by estimating the likelihood that each individual belongs to one or more of K populations (Pritchard *et al*., 2000). While robust and widely accepted, STRUCTURE is computationally intensive and becomes impractical for datasets with tens of thousands of SNPs or hundreds of individuals. In response to this limitation, fastSTRUCTURE was developed using a variational Bayesian framework that delivers similar clustering results with significantly reduced runtime. It is more suitable for fast, large-scale SNP data analysis in plants (Raj *et al*., 2014).

Nonetheless, STRUCTURE remains highly relevant and is frequently used in high-density SNP studies where analytical precision and model flexibility are prioritized, as its complete Bayesian Markov Chain Monte Carlo (MCMC) approach enables a deeper exploration of complex genetic patterns, offering superior performance in detecting subtle population structures, admixture, and scenarios involving small sample sizes, polyploidy, or non-random mating (Porras-Hurtado *et al*., 2013).

The STRUCTURE software requires specifically formatted input files, which presents a significant practical challenge in modern SNP-based studies. STRUCTURE expects genotype data to be organized in a strict text-based format where two rows and markers representing each individual are consistently encoded. These requirements are difficult to fulfill manually, especially when working with large datasets in PLINK-derived *.ped* and *.map* formats. This preprocessing step often becomes a technical bottleneck in research workflows, particularly when dealing with high-density SNP data.

To address this limitation, we introduce SNP2STR, a user-friendly Python-based tool designed to automatically convert SNP datasets from PLINK's *.ped* and *.map* formats into the input format required by STRUCTURE. SNP2STR efficiently arranges the data into a compatible matrix format, optionally includes marker names in the first row, and ensures that each individual is represented by two correctly formatted rows corresponding to allele calls. By automating this conversion process, SNP2STR removes a significant barrier to the effective use of STRUCTURE, facilitating its application in plant population genetics even when working with extensive SNP datasets.

This paper presents the rationale behind SNP2STR, describes its core functionalities, and demonstrates its utility in enabling efficient and accurate population structure analysis with STRUCTURE in the context of high-density plant SNP data.

MATERIALS AND METHODS

Data

The data used in this study was previously published by Konjić *et al*. (2023) and consists of traditional apple accessions from the Bosnia and Herzegovina apple core collection genotyped with the Axiom® Apple 480K SNP array (Bianco *et al*., 2016).

Input files

The software was developed to process *.map* and *.ped* input files (Figure 1). In this study we used Axiom data *.map* and *.ped* and files were generated using Axiom Analysis Suite (Affymetrix, Inc., version 4.0.3) from raw .CEL files.

```
#Sample Filename    Genotypes
MD Amerikanka.CEL   A A T C T T G G A A C C G G T T
MD Ananas.CEL   C C T C C C G G A A T C G G T C T T
MD Babovaca.CEL A A T C T T G G G G T T A G T T T G
MD Bascenka.CEL A A T C T T G G G G T T A A T T G G
```

A

```
MDC000001.124   AX-115533715    0.000   7112
MDC000001.124   AX-115533717    0.000   7810
MDC000001.124   AX-115533718    0.000   7922
MDC000001.124   AX-115533719    0.000   8069
MDC000002.328   AX-115533721    0.000   941
```

B

Figure 1. (A) Example of a space delimited *.ped* file containing mandatory six columns (Family ID, Individual ID, Paternal ID, Maternal ID, Sex (1=male; 2=female; other=unknown), Phenotype). The remaining columns contain genotypes (B) Example of a *.map* file where each line describes a single marker and has exactly four columns (chromosome, rs# or snp identifier, genetic distance (morgans), base-pair position (bp units)

Software development:
The SNP2STRtool was developed in Python ver. 3.6 (Van Rossum and Drake, 1995) as a command-line application for converting SNP genotype data from PED format to STRUCTURE-compatible format. The software utilizes the pandas library ver. 2.2.3 (McKinney, 2010) for efficient data manipulation and CSV processing of genomic datasets. The tool is implemented as a Python package using setuptools for distribution, with the core processing logic contained in a single module that handles file parsing, genotype conversion, and output formatting. The software includes comprehensive error handling for common data inconsistencies such as varying sequence lengths across samples and mismatched population data. The package was designed following standard Python packaging conventions with a command-line interface.

Software implementation
SNP2STR is a Python package designed to prepare SNP files for analysis within the STRUCTURE software. The script has one mandatory argument: the path to the *.ped* file that needs to be modified. Moreover, the user can optionally specify a *.map* file and a *.txt* file with the list of population identifiers.
The script produces a single output file. This is a *.txt* file with values separated by a single space. The file header, if present, shows the names of chromosomes. Each variety has two rows of sequences, one for each strand. Bases are encoded to align with the required STRUCTURE format.
The user can choose to omit the header in the output file. Note that if no *.map* file is specified, the header will be omitted by default.
To produce the output, SNP2STR reads the *.ped* file line by line. For each variety, it extracts the base sequence and encodes the into numerical format (ATGC -> 1234), missing data is represented by -9 (Pritchard *et al.*, 2009). The software then splits the sequence into two strands by taking alternating values. For example, the first row contains the first, third, and fifth values of the *.ped* sequence. The second row contains the second, fourth, and sixth values.

RESULTS AND DISCUSSION

The generated software package is hosted on the Python Package Index (PyPI) and can be installed with the pip tool (Figure 2.)

```
pip install snp2str
```

Figure 2. Example of how to install SNP"STR software with the pip tool

The software is also available on GitHub [https://github.com/vlad-ds/snp2str].

The script is operated through a command line interface (Figure 3)

```
Welcome to snp2str!
usage: snp2str [-h] [--output OUTPUT] [--skip-header] [files [files ...]]

Convert PED files to create input format for structure.

positional arguments:
  files           List paths for .ped, .map and pop.txt file. If omitted, will search for default files in current folder.

optional arguments:
  -h, --help      show this help message and exit
  --output OUTPUT  Path for the output file. Defaults to ./output.csv
  --skip-header   Skip header row in output file.
```

Figure 3. Software interface

Software can be run in python and also from Windows Powershell in Windows OS and terminal in Linux OS.

Performance

In order to test the software a dataset containing 64 apple accessions from the *ex situ* site in Srebrenik, Bosnia and Herzegovina were used. The accessions have been genotyped with the Axiom® Apple480K SNP array (Bianco *et al*., 2016). Dataset was available in *.map* and *.ped* format which were then used as input files for SNP2STR software (Figure 4).

```
# Basic usage with a PED file
snp2str path/to/your/file.ped

# Using all optional files
snp2str path/to/your/file.ped path/to/your/populations.txt path/to/your/file.map

# Specify output path
snp2str path/to/your/file.ped --output custom_output.csv

# Skip header in output file
snp2str path/to/your/file.ped --skip-output-header

# Skip first line in input PED file
snp2str path/to/your/file.ped --skip-input-header
```

Figure 4. Command line interface when running the software

The output file (Figure 5) is written as follows. First, if present in the file, the header is added, then the rows are written. Each population label gets two rows, one for each strand. The first value of each row is the population label. The second value is a population identifier if specified in the *.txt* file, otherwise, this value is omitted. The subsequent values are the base numbers for each chromosome on that strand.

```
Amerikanka.CEL 2 3 1 4 3 2 3 1 3 1 2 3 4 1 2 3 2 1 4 4 2 1 2 3 2 1 2 1 2 3 1 2 1 2 1 1 4 1
Amerikanka.CEL 2 3 1 4 3 2 3 1 3 1 4 3 4 3 2 3 3 3 4 4 4 1 2 3 2 3 4 1 4 3 3 2 1 2 3 1 4 3
Ananas.CEL 4 3 1 2 3 2 2 1 1 -9 2 1 2 1 2 1 3 1 4 2 4 1 2 2 2 3 4 1 4 1 3 2 1 2 3 1 4 1 2
Ananas.CEL 4 3 1 4 3 4 2 3 3 -9 2 1 4 1 4 3 3 3 4 4 4 3 2 3 2 3 4 1 4 3 3 3 1 4 3 3 4 1 3
Babovaca.CEL 2 3 3 2 1 2 2 1 3 1 2 1 2 1 2 3 2 1 4 2 4 1 2 2 2 1 4 1 2 1 1 2 3 4 1 3 2 1 3
Babovaca.CEL 2 3 3 2 3 2 3 1 3 1 2 3 4 1 2 3 3 3 4 4 4 3 3 3 2 3 4 1 2 3 1 3 3 4 3 3 4 1 3
Bascenka.CEL 2 3 3 2 1 2 3 1 3 1 2 3 2 1 2 3 3 3 4 2 4 1 2 3 2 1 4 1 2 1 1 2 3 4 3 3 2 1 3
Bascenka.CEL 2 3 3 2 1 2 3 1 3 3 2 3 2 1 4 3 3 3 4 2 4 1 3 3 2 1 4 1 2 3 1 2 3 4 3 3 2 1 3
Bedrika.CEL 2 2 1 2 1 2 2 1 3 1 2 1 -9 1 2 3 3 1 4 2 2 1 2 2 2 3 4 1 2 1 1 3 3 4 1 1 2 1 2
Bedrika.CEL 2 3 3 2 3 4 3 1 3 3 2 3 -9 1 2 3 3 3 4 2 4 1 2 2 4 3 4 3 4 3 1 3 3 4 3 3 4 1 3
Bihorka.CEL 2 3 1 2 3 2 3 1 3 1 2 3 2 1 2 1 2 1 4 2 4 1 2 3 2 1 2 1 2 1 1 2 3 2 1 1 2 1 2
Bihorka.CEL 2 3 3 4 3 2 3 3 3 3 4 3 4 3 4 3 2 3 4 2 4 1 2 3 2 3 4 3 2 3 1 2 3 2 3 3 4 3 3
Bistrica.CEL 2 3 1 2 3 2 3 1 -9 1 2 1 4 1 2 3 2 1 4 2 2 1 2 2 2 1 4 1 2 1 1 3 3 2 1 1 4 1
Bistrica.CEL 2 3 3 2 3 2 3 1 -9 3 2 3 4 1 2 3 3 3 4 4 4 1 2 3 4 3 4 1 4 3 1 3 3 4 3 3 4 1
```

Figure 5. Input format for STRUCTURE software after conducting snp2str

To our knowledge conversion to structure format can also be done with PGD spider (Lischer & Excoffier 2012) and Rstudio (R Studio Team 2020). However, in trying to convert the *.vcf* file to STRUCTURE format in PGD spider, it yielded a table with distorted numbers. The cause of this issue is unclear and may be due to format incompatibility between the .vcf file and PGDSpider's input requirements, a software bug, or errors in the conversion settings or process. As far as Rstudio is concerned, the operation would involve a few different steps and a range of skills in the program.

These limitations highlight the need for a stand-alone, scriptable solution that eliminates manual reformatting and conversion errors—precisely the gap that SNP2STR is designed to fill.

Even though STRUCTURE software is widely known and popular when it comes to population structure analysis, it takes too long to analyse large datasets containing SNPs (Raj *et al.,* 2014). Therefore, a new software named fastStructure (Raj *et al*., 2014) was designed specifically for inferring population structure from SNP data. As such, this software accepts outputs derived in PLINK as inputs, but it also accepts files in the 'classical' STRUCTURE format.

However, STRUCTURE software analysis remains highly relevant and is used in high-density SNP studies where analytical precision and model flexibility are prioritized. This makes it especially valuable in detecting subtle population structures, admixture, and scenarios involving small sample sizes, polyploidy, or non-random mating—cases where a more nuanced analysis is essential (Porras-Hurtado *et al*., 2013). Additionally, STRUCTURE offers advanced modeling capabilities such as the LOCPRIOR model, correlated allele frequencies, and detailed ancestry estimation, features not fully supported by fastSTRUCTURE. These qualities make STRUCTURE the preferred choice in studies requiring publication-standard resolution or methodological benchmarking, even if it necessitates using a subset of SNPs or longer computational time.

In this context, SNP2STR remains a valuable tool for facilitating data preparation, enabling researchers to efficiently utilize the method best suited to their dataset size and research goals. The main advantage of SNP2STR lies in its straightforward interface

and ability to streamline the conversion of SNP datasets into STRUCTURE-compatible formats, reducing manual effort and potential formatting errors
Overall, the value of the newly developed software lies in its simple user interface, which clearly saves time in the analysis process. The tool has been validated on dataset from Axiom® Apple 480 K SNP array (Bianco *et al.*, 2017), and so far no limitations have been observed.

Future developments
Future development efforts are focused on expanding the software's compatibility with additional input formats. Specifically, future updates aim to support direct processing of Variant Call Format (*.vcf*) files, thereby streamlining the workflow for users working with raw genomic data.

CONCLUSIONS

High-density SNP datasets have revolutionised plant population genetics, but the strict input format required by STRUCTURE remains a technical bottleneck. SNP2STR removes this hurdle by converting .ped and .map files into STRUCTURE-ready matrices in a single command. Testing on a 64-accession apple dataset showed that the script produces fully compliant output in seconds and without user intervention.

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SNP2STR: SOFTVER ZA KREIRANJE INPUT DATOTEKA ZA STRUCTURE SOFTVER IZ SNP PODATAKA

Sažetak

STRUCTURE je softverski paket koji koristi molekularne podatke iz više lokusa za analizu strukture populacije. Naime, Za analizu podataka zasnovanih na jednonukleotidnim polimorfizmima (engl. *Single Nucleotide Polymorphism* – SNP) pomoću softvera STRUCTURE, potreban je specifičan format ulazne datoteke. Međutim, budući da SNP studije često obuhvataju hiljade lokusa, takve datoteke obično su prevelike za manuelno kreiranje.

Kako bi se prevazišao ovaj izazov, razvijeni su različiti softverski alati i funkcije. Mešutim, kada je riječ o biljnim podacima, neki od tih alata ne uspijevaju ispravno obraditi podatke ili generišu netačne ulazne datoteke.

Kako bi se adresirali navedeni problemi razvijen je novi softverski alat — SNP2STR. SNP2STR vrši pretvaranje SNP podataka u input format koji zahtjeva STRUCTURE softver. Ovaj softver generiše datoteku koja sadrži nazive markera, sa po dva reda po jedinki formatirana u skladu sa pripadajućim markerima.

Softver je open-source i dostupan na GitHub-u. Također nudi potencijal za buduća unapređenja na osnovu povratnih informacija korisnika.

Ključne riječi: *molekularni podaci, analiza strukture populacije, genetički clustering, open-source bioinformatički alati*

ASSESSMENT OF PHYSICAL AND CHEMICAL SOIL PROPERTIES IN URBAN GREEN AREAS OF NOVI GRAD MUNICIPALITY, SARAJEVO CANTON*

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Preliminary communication

Abstract

Modern urbanization trends are increasing population density in cities and suburban areas, posing challenges to environmental quality. Urban soils play a crucial role in supporting ecological functions such as water filtration, carbon storage, and biodiversity maintenance. Despite this, they are often subject to anthropogenic pressures that degrade their structure and functionality. While soil research has traditionally focused on agricultural and forest lands, urban soils-particularly in Bosnia and Herzegovina-remain understudied, especially regarding contamination by heavy metals and other pollutants.

This study investigates the condition of soils at three urban green locations in the Novi Grad municipality, Sarajevo Canton, under different land uses (lawn, tree-covered, arable). The results show that soils are predominantly sandy and carbonate, with slightly alkaline pH. Humus content ranges from 3.3 to 4.19%, while carbon stocks vary from 6.8 kg/m² (lawn) to 8.1 kg/m² (tree-covered areas). The soils are moderately to well supplied with nitrogen (average C/N ratio of 13:1), moderately supplied with available potassium, and poorly supplied with available phosphorus. Filtration properties are generally poor to moderate, with an average water retention capacity of 43.96% vol.

Importantly, all soil samples contained heavy metals below harmful concentration thresholds, as defined by national standards. These findings can inform improved urban soil management strategies in the Sarajevo Canton.

Keywords: *urban soils, soil ecosystem services, environmental quality, carbon storage, soil contamination*

INTRODUCTION

Modern trends reveal an increasing population in urban and suburban areas, presenting numerous challenges for maintaining environmental quality in these settings (Pavao-Zuckerman, 2008; Guilland *et al*., 2018). Due to rapid urbanization, soils in urban areas, often referred to as Urbisols, become a crucial component of urban ecosystems, serving various ecological functions. These soils are formed through intensive anthropogenic

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activities that disrupt or entirely remove natural soil horizons, leading to the presence of distinct layers brought in from outside sources. Urban soils provide essential ecosystem services, such as storing soil organic carbon (SOC), filtering and retaining water—an essential function for mitigating urban floods-and supporting biomass production and biodiversity maintenance (Yang & Zhang, 2015; Pavao-Zuckerman, 2012). Urban soils also play a role in ecological restoration, where a thorough understanding of their functions can guide successful outcomes (Pavao-Zuckerman, 2012).

Nonetheless, urban soils experience substantial pressure from anthropogenic activities, which often lead to the degradation of their structure and functionality (Yang & Zhang, 2015). Traditionally, soil quality studies have focused on agricultural and forest lands, often neglecting urban soils. However, with the rise of urban agriculture and the intensifying fight against climate change, urban soils have become an increasingly important research focus. Urban soils also serve as sites for the deposition, transformation, and transfer of various substances, including contaminants, in urban-industrial zones. Additionally, as one of the largest terrestrial carbon sources, soil plays a vital role in the global carbon cycle by storing atmospheric CO_2 as soil organic matter, a critical contributor to carbon sequestration. Therefore, it is crucial to quantify the functions of urban soil and understand its role for the environment and society, especially in the context of sustainable urban development (Vasenev *et al*., 2017).

In Bosnia and Herzegovina, research on urban soil conditions remains scarce, particularly in assessing contamination by heavy metals and other pollutants in industrial urban areas. Given these issues, it is essential to quantify the functions of urban soils and understand their role in the sustainable development of cities. This research aims to measure SOC storage, assess nutrient content (N, available P_2O_5, and K_2O) related to soil fertility and biodiversity conservation, evaluate water holding capacity and permeability, and analyze heavy metal concentrations in urban soils, which will give insights into urban ecosystem resilience. The research aims to assess the capacity of urban green spaces in Novi Grad Municipality to store carbon in soil, support biomass production and biodiversity, retain water to prevent flooding, and mitigate pollution by absorbing heavy metals. Given the rarity of such studies in Bosnia and Herzegovina, this research will analyze different types of urban green areas - lawns, cultivated land, and tree-covered zones - to determine their impact on urban soil properties and ecosystem functions.

MATERIAL AND METHODS

The study area includes three locations in the Novi Grad municipality of Sarajevo Canton: the "Safet Zajko" Sports (SRC „SZ“) and Recreation Center (43.8477° N, 18.3365° E), "Mojmilo" Park-Forest (43.8359° N, 18.3575° E), and the Flora Nursery in Nedžarići (43.8346° N, 18.3309° E). The SRC "SZ" offers sports and recreational facilities, the "Mojmilo" Park-Forest provides walking trails, and the Flora Nursery is a key site for growing ornamental plants distributed to other green spaces in Sarajevo

Canton. Three places in each mentioned location (about 30 cm deep) were dug, representing different land uses:

1. SRC „SZ“: grassland with mixture of grass, cultivated soil for ornamental plants - roses and deciduous trees covered area;
2. Park-Forest: grassland with mixture of grass, cultivated soil under shrubbery and deciduous trees covered area;
3. Flora Nursery: grassland with mixture of grass, cultivated soil under fruit nursery and deciduous trees and shrubs covered area;

Each location provides unique conditions and resources for research, contributing to a deeper understanding of the ecological and biological aspects of soils in Novi Grad and the Sarajevo Canton. The Safet Zajko Sports and Recreation Center and the Mojmilo Park-Forest were selected due to their significance as popular urban green spaces frequently used for recreational activities by the citizens of Sarajevo. In contrast, the Flora Nursery was chosen because it is a managed and maintained urban area, serving as a representative example of how urban soils can be systematically utilized and maintained, offering valuable insights into soil management practices in urban environments.

It should be emphasized that the SRC “SZ” and the “Flora” nursery are located on alluvial deposits with the Miljacka and Dobrinja rivers nearby. The “Mojmilo” Forest Park belongs to a mountainous area. The SRC “SZ” and the “Flora” nursery are located on lower exposures of the relief, on flattened alluvial deposits with rivers in the immediate vicinity of the Miljacka (SRC “SZ”) and Dobrinja rivers (the “Flora” nursery). The third research location in this paper, the “Mojmilo” Forest Park in the context of the described relief, is located on the southern edge of the Canton in a transitional mountainous area, i.e. Mojmilo Hill.

In March, site visits determined that these three locations are the most suitable for research, as they are close to one another and offer diverse land uses within the same geographical and climatic area under similar physical and chemical soil characteristics. Soil sampling for physical-mechanical and chemical analysis took place from May 8 to May 23, 2024. At each site, three pits (about 30 cm deep) were dug, representing different land uses: grassland, cultivated soil, and tree-covered area, totaling nine pits. Samples were taken from depths of 0–15 cm and 15–30 cm in disturbed and undisturbed states, with three replicates each. The paper used mean values from three analyses.

A soil samples were collected for physical, hydropedological and chemical properties, including texture, bulk density, permeability, water holding capacity, pH, soil organic carbon (C) content, nutrient levels (N, P, K), and heavy metal concentrations (Table 1).

Table 1. All the analyzes that were done in the laboratory

Category	Method	Reference
Soil Organic Matter	Determination of C and humus content by colorimetric method	ISO 14235:1994
	Determination of soil carbon content (kg/m²)	Hengl *et al.* (2018)
	Determination of total nitrogen using Kjeldahl method and C/N ratio	ISO 11261
Physical Properties	Determination of soil texture	Škorić (1982)
	Determination of soil structure according to Sekeri	Resulović *et al.* (1982)
	Determination of bulk density (BD)	ISO 11272
Water-Physical Properties	Determination of hygroscopic moisture by Mitscherlich method	-
	Determination of current moisture and soil water retention capacity	Gračanin (1977)
	Determination of filtration coefficient	Eijkelkamp
Soil Chemical Properties	Determination of carbonate content ($CaCO_3$)	ISO 10693:2015
	Determination of soil pH reaction	ISO 10390:2005
	Determination of available phosphorus and potassium by AL method	Resulović *et al.* (1982)
	Determination of heavy metal concentrations using AAS	-

To determine the maximum permissible values for heavy metal content, the table from Rulebook on determining permissible quantities of harmful and hazardous substances in soil and methods of their testing from Službene novine Federacije Bosne i Hercegovine (2022), which is used in our country (Table 2):

Table 2. Limit total values of pollutant content *

Heavy metals	Limit values by texture (mg kg^{-1})		
	Sandy	Silty and loam	Clayly
Cadmium (Cd)	0.5	1	1.5
Copper (Cu)	50	65	80
Nickel (Ni)	30	40	50
Lead (Pb)	50	80	100
Zinc (Zn)	100	150	200
Chromium (Cr)	50	80	100
Cobalt (Co)	30	45	60

*[16], the stated values refer to soils with an acidic reaction. In carbonate soils, the threshold for limit values can be increased by 25%

RESULTS AND DISSCUSSION

The results in table 2 shows that carbon storage values for grass-covered areas in SRC "SZ" and Park-Mojmilo range between 5.8-6.4 kg/m² (to 30 cm depth), while at Flora Nursery, it reaches approximately 8.4 kg/m². Cultivated areas show slightly higher carbon storage, ranging from 5.9-8.2 kg/m². Tree-covered areas vary significantly, with carbon storage values of 5.5-6.1 kg/m² at the first two sites, but around 12 kg/m² at Flora Nursery. Also, the BD for alluvial soils in Great Britain was 0.92 g/cm³ (0.22 - 1.52 g/cm ³) (Edmondson *et al.,* 2012) which is comparable to the results in this paper. Building on the previously mentioned fact that the SRC "Safet Zajko" and the Flora Nursery are located on alluvial deposits formed by the nearby Miljacka and Dobrinja rivers, the observed soil properties-particularly bulk density-can be meaningfully compared to those of similar soils. For example, the bulk density (BD) for alluvial soils in Great Britain was reported as 0.92 g/cm³ (range: 0.22-1.52 g/cm³) (Edmondson *et al.*, 2012), which aligns with the values recorded at these sites in this study.

Table 3. Values for Bulk density, SOM, C%, and carbon stock for the locations

Parameters	Bulk density (g/cm^3)		Soil organic matter (SOM) (%)		C (%)		Carbon stock (kg/m^2)
Depth (cm)	0-15	15-30	0-15	15-30	0-15	15-30	0-30
Grassland							
SRC „SZ“	1.17	1.29	3.05	2.42	1.77	1.41	5.83
Park forest Mojmilo	1.31	1.29	3.05	2.60	1.78	1.51	6.41
Nursery „Flora“	1.04	1.19	4.53	4.15	2.64	2.41	8.41
Cultivated soil							
SRC „SZ“	0.99	0.93	4.20	5.64	2.44	3.28	8.23
Park forest Mojmilo	1.13	1.20	3.60	2.26	2.09	1.31	5.90
Nursery „Flora“	1.06	1.06	4.33	4.25	2.52	2.47	7.93
Soil under trees							
SRC „SZ“	1.35	1.30	2.86	2.50	1.66	1.45	6.17
Park forest Mojmilo	1.19	1.34	2.23	2.81	1.30	1.63	5.58
Nursery „Flora“	1.22	1.15	8.43	6.32	4.90	3.67	12.47

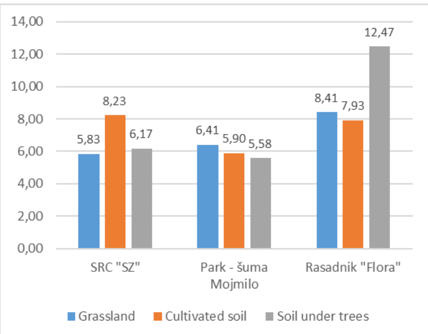

Figure 1. Carbon stock under 30 cm

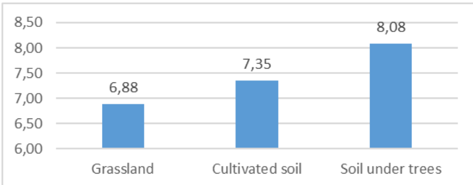

Figure 2. X̄ of carbon stock from different land cover

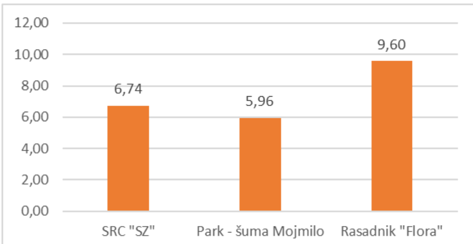

Figure 3. X̄ of carobon stock for locations

Thus, Flora Nursery's soils are the most optimized for carbon storage through careful management and occasional fertilization, averaging around 9.6 kg/m², while SRC "SZ" and Park-Mojmilo range between 5.9 and 6.7 kg/m². The highest carbon storage values are associated with tree-covered areas, averaging 8.0 kg/m² across all three locations, compared to cultivated and grass-covered areas with averages of 6.8 and 7.3 kg/m², respectively (fig. 2 and 3).
Analyzing percentage of C (table 3) per land use type, grass-covered areas at SRC "SZ" and Park-Mojmilo have 1.5-1.7%, while Flora Nursery's grass areas hold slightly higher values around 2.5% due to less disturbance and occasional fertilization. Cultivated soils show C% above 2% in the top 15 cm, with varying values at 15-30 cm, while tree-covered soils at Flora Nursery contain the highest %C values (nearly 5% in the top 15 cm, and 4% at 15–30 cm). These values are likely due to the coniferous trees at Flora, which contribute higher organic input through needle fall.
Comparing these results with Gao *et al.* (2021), Zhang & Xu (2013), and Luo *et al.* (2015), who reported an average of 3.5 kg/m² carbon storage (up to 30 cm depth), it is evident that areas in Novi Grad exhibit higher carbon storage levels than those found in the literature. In their work, Tvica and Čustović (2010) (Table 4) analyzed the carbon stock in sandy loam in Nišići and loam in Sprečko polje in dominant soil type in Bosnia with similar texture and climatic conditions and found the following soil organic carbon stock in arable soils and soils under grass vegetation and forest.

Table 4. SOC stock under 30 cm depth (Tvica i Čustović, 2010)

Stock of SOC under 30 cm depth	Nišići - Dystric cambisol, sandy loam			Sprečko polje – Pseudogley, loam		
	Arable and	Grass	Forest	Arable land	Grass	Forest
kg/m^2	5.46	4.76	6.56	5.21	8.14	8.42

Similarly, in their research on the soil organic carbon stock in forest nurseries and in the nearby soil under grass vegetation in central Bosnia - Žepče (similar soil and climatic condition), up to 30 cm depth, Tvica *et al*. (2013) finds 7.57 kg/m^2 SOC in grassland and slightly less, 5.83 kg/m^2 SOC in the forest nursery itself.

Table 5. Values of N, K_2O, P_2O_5 , carbonate content and pH for the locations under different land cover

Location	Land cover	Depth (cm)	pH in 1M KCl	W($CaCO_3$) (%)	N (%)	K_2O (mg/100g)	P_2O_5 (mg/100g)
SRC „SZ“	Grassland	0-15	7.57	8.83	0.175	10.6	5.3
		15-30	7.57	8.09	0.179	19.7	4.6
	Cultivated soil	0-15	7.65	11.67	0.214	14.7	6.5
		15-30	7.66	9.72	0.240	13.5	6.1
	Soil under trees	0-15	7.52	4.22	0.208	12.5	1.6
		15-30	7.51	2.57	0.182	10.8	1.2
Park forest Mojmilo	Grassland	0-15	7.30	6.45	0.220	12.0	0.0
		15-30	7.10	1.57	0.204	7.7	0.1
	Cultivated soil	0-15	7.50	8.95	0.213	12.2	1.3
		15-30	7.48	10.64	0.173	10.5	0.8
	Soil under trees	0-15	4.49	-	0.236	13.7	0.6
		15-30	4.27	-	0.174	10.1	0.0
Nursery „Flora“	Grassland	0-15	7.20	1.81	0.193	46.9	27.2
		15-30	7.29	2.84	0.176	46.9	24.4
	Cultivated soil	0-15	7.55	7.50	0.184	44.9	39.0
		15-30	7.57	8.44	0.181	35.9	37.2
	Soil under trees	0-15	7.19	3.58	0.359	46.9	23.3
		15-30	7.21	4.73	0.269	45.9	23.6

The analysis (table 5) indicates that pH and carbonate content in the soil are consistent across all locations. Lawn areas and cultivated surfaces have slightly alkaline pH values (7.2–7.5), while soil under trees is acidic, at the Mojmilo site (pH 4.2-4.4) due to the influence of coniferous trees and forest vegetation. Gračanin (1948) notes that tannins from tree roots lower soil pH, which can lead to soil acidification below pH 5.

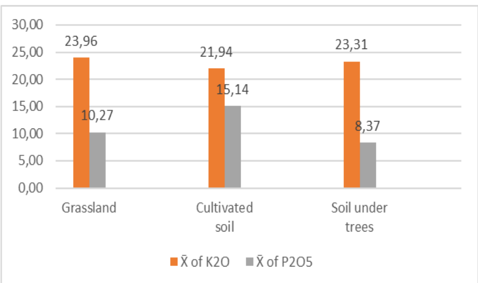

Figure 4. $\bar{X}$ for K_2O, P_2O_5 in mg/100 g soil, content in soil from different land cover

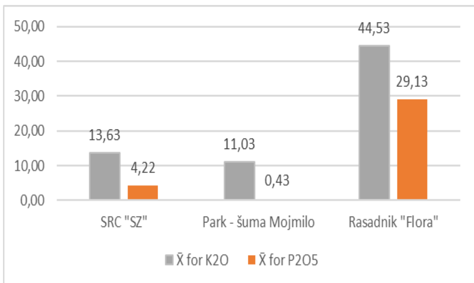

Figure 5. $\bar{X}$ for, K_2O, P_2O_5 content in soil for locations

The site "Flora" has a high supply of potassium and phosphorus, attributed to fertilization, while other sites have lower nutrient availability (fig. 4 and fig. 5). Pouyat *et al*. (2006) states that urban soils often contain elevated potassium and phosphorus concentrations due to artificial fertilizers, which is also evident at the "Flora" site.

Table 6. Soil texture according to the FAO triangle for determining soil texture

Land cover	**Grassland**		**Cultivated Soil**		**Soil under Trees**	
Depth	0 - 15 cm	15 - 30 cm	0 - 15 cm	15 - 30 cm	0 - 15 cm	15 - 30 cm
SRC "SZ"	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Loam	Loam
Forestry park Mojmilo	Sand	Sand	Sandy loam	Sandy loam	Loam	Clay loam
Nussery "Flora"	Sandy loam	Loamy sand	Loamy sand	Loamy sand	Loamy sand	Loamy sand

The dominant soil textures (table 6) for the sites are as follows: SRC "SZ" is mostly sandy loam, with loam under trees; the second location has sand in the lawn area, sandy loam in cultivated land, and loam or clay loam under trees. The third location predominantly features loamy sand, with sandy loam in the 0-15 cm layer under lawn areas. This composition reflects the historical deposition of river materials in the Sarajevo Basin, resulting in soils with high sand, silt, and clay content.

Table 7. Values of flitration (permeability) for the locations under different land cover

Land cover	Grassland		Cultivated soil		Soil under trees	
	m/day		m/day		m/day	
Depth (cm)	0-15	15-30	0-15	15-30	0-15	15-30
SRC“SZ“	0.068261	0.035334	0.452130	0.079630	0.024009	0.540531
Permeability mark	Low	Low	Medium	Low	Low	Medium
Park forest Mojmilo	0.422614	0.189147	0.047656	0.072504	0.334629	0.082081
Permeability mark	Medium	Medium	Low	Low	Medium	Low
Nursery“Flora“	0.130147	0.003770	0.162732	0.111569	0.056239	0.012632
Permeability mark	Medium	Very low	Medium	Medium	Low	Low

The filtration coefficient values in table 7 indicate that most soil samples exhibit low permeability according to the classification values of Vukašinović (1956). Turf areas mostly show low permeability at the first location, medium at the second and third locations, with the third location at 15-30 cm depth showing very low permeability. Cultivated areas have medium permeability at the first and third locations, while the second location has low permeability. Tree-covered areas exhibit low permeability at the first and third locations, with exceptions at 15-30 cm depth at SRC "SZ" and at 0-15 cm depth at Park-forest Mojmilo. The second location ranges from medium to low permeability.

Table 8. Values of water holding capacity for the locations under different land cover

Land Cover	Grassland		Cultivated soil		Soil under trees		Average (location)
	%vol		%vol		%vol		%vol
Depth (cm)	0-15	15-30	0-15	0-15	0-15	15-30	
SRC“SZ“	42.6	41.63	39.73	46.50	42.47	39.05	42.00
Park forestry Mojmilo	45.27	41.17	47.77	44.93	42.70	41.77	43.93
Nursery Flora	48.9	45.77	44.23	45.10	45.67	46.20	45.98
Average (land cover)	44.22		44.71		42.97		43.97

The water holding capacity values in table 8 for all three locations under turf surfaces are above 40% vol., with the highest values at the Flora nursery reaching up to 48.9% vol. Cultivated areas range from 39.7-47.7% vol., showing notable depth-related differences at the first location (39.7% vol. at 0-15 cm and 46.5% vol. at 15-30 cm). Tree-covered areas have similar values, with a depth variance of around 3% vol. Average retention moisture by land use type ranges from 42.97% vol. (trees) to 44.71% vol. (cultivated), and by location, from 42-45.9% vol.

Table 9. Values of heavy metals (ppm) according to land use methods

Location	**SRC "SZ"**					
Land cover	Grassland		Cultivated Soil		Soil under Trees	
Depth	0 - 15 cm	15 - 30 cm	0 - 15 cm	15 - 30 cm	0 - 15 cm	15 - 30 cm
Cu	10.93	9.08	22.23	24.66	11.32	12.98
Zn	40.07	41.29	43.64	44.15	33.6	37.61
Cd	0.05	0.04	0.03	0.03	0.04	0.04
Pb	17.42	21.77	30.33	27.86	15.53	21.12
Ni	22.73	24.26	30.64	25.66	34.77	41.21
Cr	2.38	21.54	17.3	17.26	21.63	24.31

Location	**Forestry park Mojmilo**					
Land cover	Grassland		Cultivated Soil		Soil under Trees	
Depth	0 - 15 cm	15 - 30 cm	0 - 15 cm	15 - 30 cm	0 - 15 cm	15 - 30 cm
Cu	4.29	4.40	23.76	16.59	3.44	4.91
Zn	31.09	32.98	37.39	34.65	29.63	36.47
Cd	0.06	0.05	0.05	0.04	0.05	0.05
Pb	15.09	21.62	20.61	19.74	24.38	31.64
Ni	35.83	49.61	27.97	36.51	27.11	37.11
Cr	0.44	18.72	29.4	30.55	29.49	31.94

Location	**Nussery “Flora”**					
Land cover	Grassland		Cultivated Soil		Soil under Trees	
Depth	0 - 15 cm	15 - 30 cm	0 - 15 cm	15 - 30 cm	0 - 15 cm	15 - 30 cm
Cu	7.80	9.89	8.85	11.16	13.29	15.14
Zn	39.91	42.46	45.34	47.06	43.24	44.42
Cd	0.04	0.03	0.04	0.04	0.03	0.03
Pb	8.13	16.84	13.35	19.16	16.69	20.61
Ni	0.62	26.66	24.83	26.26	24.29	27.17
Cr	0.35	17.75	21.37	20.31	17.22	20.13

Additionally, all soil samples contain heavy metals at concentrations below the threshold values considered harmful (table 9), as defined by the official guidelines of the Official Gazette of the Federation of Bosnia and Herzegovina (Službene novine Federacije Bosne i Hercegovine, 2022).

CONCLUSIONS

The study results indicate that carbon stocks up to 30 cm of soil depth vary across the three locations, with an average value of 7.44 kg C/m², reaching the highest levels (8.08 kg C/m²) under tree-covered soils. Similarly, water holding capacity averaged 43.97% vol, while permeability ranged from low to medium intensity. The C/N ratio averaged 10.6 : 1. Available phosphorus and potassium levels varied from low to high, influenced by the fertilization practices of green areas. Notably, the concentrations of heavy metals were within permitted limits at all locations.

However, it is evident that results differ significantly between locations, primarily due to the notably higher values recorded at the "Flora" site. This discrepancy is likely due to the historically high soil quality at this particular location, combined with good land management practices and the influence of fertile fluvial soil from the Dobrinja River, making direct comparisons between locations challenging.

These findings have important practical implications for the management and sustainability of urban green spaces. The results emphasize the significant role of urban green areas in storing carbon, contributing to climate change mitigation through soil organic matter. Additionally, the study highlights the importance of green spaces in water retention, which is crucial for regulating the local water cycle, particularly in the context of increasing urbanization and extreme weather events.

However, the study also reveals limitations in the permeability and filtration capacity of these areas, which could hinder their potential role in flood prevention. While water retention is effective, further improvements in filtration processes may be necessary to fully optimize the ecological functions of urban green spaces in flood-prone areas. The variation in soil quality and ecological functions between locations, especially the superior results at the "Flora" site, underscores the importance of site-specific management practices. The high soil quality at "Flora" demonstrates how good land management, combined with favorable environmental factors, can significantly enhance the ecological functions of urban green spaces. Therefore, "Flora" can serve as a model for effective green space management in Novi Grad Municipality.

In conclusion, the study underscores the value of urban green spaces, particularly the "Flora" nursery, in supporting ecological functions such as carbon sequestration, biodiversity conservation, and water retention. Nevertheless, to optimize their role in urban flood management, future strategies should focus on enhancing soil permeability and filtration capacity. These findings provide valuable insights for sustainable urban planning and the continued improvement of green spaces in urban areas.

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PROCJENA FIZIČKIH I KEMIJSKIH SVOJSTAVA TLA U URBANIM ZELENIM POVRŠINAMA OPĆINE NOVI GRAD, KANTON SARAJEVO

Sažetak

Savremeni trendovi urbanizacije povećavaju gustoću naseljenosti u gradovima i prigradskim područjima, što predstavlja izazov za kvalitetu okoliša. Urbana tla igraju ključnu ulogu u podržavanju ekoloških funkcija poput filtracije vode, skladištenja ugljika i održavanja bioraznolikosti. Uprkos tome, često su izložena antropogenim pritiscima koji degradiraju njihovu strukturu i funkcionalnost. Dok su se istraživanja tla tradicionalno usredotočila na poljoprivredno i šumsko zemljište, urbana tla - posebno u Bosni i Hercegovini - ostaju nedovoljno proučena, posebno u pogledu onečišćenja teškim metalima i drugim zagađivačima. Ova studija istražuje stanje tla na tri urbane zelene lokacije u općini Novi Grad, Kanton Sarajevo, pod različitim načinima korištenja zemljišta (travnjaci, šumovitost, obradivo zemljište). Rezultati pokazuju da su tla pretežno pjeskovita i karbonatna, s blago alkalnim pH. Sadržaj humusa kreće se od 3,3 do 4,19%, dok zalihe ugljika variraju od 6,8 kg/m² (travnjak) do 8,1 kg/m² (područja prekrivena drvećem). Tla su umjereno dobro opskrbljena dušikom (prosječan omjer C/N od 13:1), umjereno opskrbljena dostupnim kalijem i slabo opskrbljena dostupnim fosforom. Svojstva filtracije su općenito loša do umjerena, s prosječnim kapacitetom zadržavanja vode od 43,96% vol.Važno je napomenuti da su svi uzorci tla sadržavali teške metale ispod štetnih pragova koncentracije, kako je definirano nacionalnim standardima. Ovi nalazi mogu informisati o poboljšanim strategijama upravljanja urbanim tlom u Kantonu Sarajevo.

Ključne riječi: *urbana tla, usluge ekosustava tla, kvaliteta okoliša, skladištenje ugljika, onečišćenje tla*

CONTROL OF CODLING MOTH (*CYDIA POMONELLA* L.) BY APPLICATION OF INSECTICIDE CHLORANTRANILIPROLE*

Slavica Vuković[1], Antonije Žunić[1], Dragana Šunjka*[1], Sanja Lazić[1], Miloš Petrović[1], Nikola Laćarac[1]

Original scientific paper

Abstract

In the last few decades, intensive apple farming has required increasing technology and knowledge to ensure a high yield and product quality. In pest and disease management in apple orchards, in addition to various control tools including agrotechnical and biological ways of protection, it is necessary to apply different chemical pesticides for plant protection several times during the year. The codling moth is one of the most important orchard pests, particularly in apples. It often causes high damage, resulting in premature fruit drop, larvae-eaten fruit, and difficulties in preserving fruit during storage, primarily due to untimely and unsuitable protection. In 2023 and 2024, the trials were carried out according to the standard EPPO methods. This study aimed to assess the efficacy of plant protection product based upon chlorantraniliprole (200 g a.i./L, SC), against the codling moth in apple protection. In the apple orchard (variety Idared, Golden Delicious and Gala) at Budisava and Mala Remeta localities (Vojvodina, Serbia), the product were foliar applied by backpack sprayer at a concentration of 0.02%. The experiment was set up in four replications in randomized block design. By visual examination the presence of C. pomonella eggs was registered, and the hatching of caterpillars of the first (2023) and second (2024) generation of this pest was underway. The efficacy of the tested insecticide was evaluated based on the number of damaged fruits. The efficacy of the insecticide was calculated according to Abbott, and the significance of differences (ANOVA) and LSD test for the confidence interval of 95%. In 2023, fourteen days after application the efficacy chlorantraniliprole was 95.3% at the locality Budisava, while in locality M. Remeta efficacy was 92.4%. After 22 days, the product showed good efficacy which ranged from 88.2-93.0%, depending on the locality. In 2024, 14 days after application, the efficacy was 93.7% at the locality Budisava, while in locality M. Remeta efficacy was 89.8%. After 25 days, the insecticide showed good efficacy which ranged from 89.5-90.6%, depending on the locality. In both years, the number of damaged apples in the variants where applied chlorantraniliprole was significantly lower compared to the control. In the studied localities, in bouth years, *C. pomonella* displayed notable susceptibility to chlorantraniliprole belonging to the diamides groups. This indicates that this insecticide

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can still be effectively used for managing this economically significant pest in apple orchards, as long as the anti-resistance strategy is strictly adhered to.

Keywords: *apple, Cydia pomonella, chlorantraniliprole, efficacy*

INTRODUCTION

The apple (*Malus domestica*) represents one of the most significant fruit species in the world and is certainly the most significant within its genus (Malus). Among all fruit species grown globally, the apple ranks third in production and consumption, right after citrus and bananas (Šoškić, 2011). Among the pests that attack apples, of particular importance are the *Cydia pomonella, Aphis pommi, Epicometis hirta, Melolontha melolontha, Hoplocampa testudinea, Hyphantria cunea,* and various other pests. The codling moth (*C. pomonella*) is one of the most significant pests of pome and stone fruits worldwide, and it is the most important pest on apples and pears. This species originated from Eurasia but has managed to spread to all areas where apples are grown, thanks to its adaptability. The main contributors to the spread of the codling moth were human migrations along global trade routes (Wearing *et al*., 2001). The species appears every year and causes the greatest damage to the fruit, reducing their market value and making storage difficult or even impossible (Kereši *et al*., 2019). Control and the strategy primarily depends on the population density that needs to be determined and monitored. The first measure to implement in orchards is the use of pheromone traps to monitor the presence and population density of the codling moth. Also, collecting and destroying infested fruits from the orchard, especially after June fruit thinning, is significant. Due to the frequent use of insecticides, reduced efficacy of certain insecticides has been recorded in many apple orchards. Therefore, alternating insecticides with different modes of action plays a crucial role in preventing resistance development in this pest to the most commonly used insecticides. In recent years, it often happens that maximum temperatures during spring reach up to 30 ºC. Then, the development of the codling moth is much faster, and the satisfactory efficacy of insecticides is shorter due to faster degradation. In the last few decades, intensive apple production requires increasing levels of technology and knowledge to achieve desired yields. The aim of this study was to determine the efficacy of the insecticide chlorantraniliprole, which belongs to the chemical group of diamides and has a different mode of action from most insecticides used in controlling *C. pomonella*, under field conditions.

MATERIALS AND METHODS

During 2023 and 2024, experiments were conducted in apple orchards (varieties Golden Delicious, Idared, and Gala) at the Budisava and Mala Remeta localities for the control of the codling moth (*C. pomonella*). The experiments were set up according to standard EPPO methods for experiment design and data analysis (Anonymous, 2012), insecticide efficacy for codling moth (Anonymous, 2004), and phytotoxicity (Anonymous, 2014). The product based on chlorantraniliprole (200 g a.s. /L, SC) was applied at a concentration of 0.02%. The treatment was carried out foliarly with a backpack atomizer "Solo 423", using 1000 liters of water per hectare. Immediately before the treatment, a visual inspection of the orchard recorded the presence of codling moth eggs (*C. pomonella*). Egg laying and larval hatching of the first (June 5, 2023) and second (July 6 and 8, 2024) generations of this pest were ongoing. The obtained data were statistically processed using ANOVA and LSD tests for a confidence interval of 95%, and efficacy was determined according to Abbott (Wentzel, 1963).

RESULTS AND DISCUSSION

Results of testing the insecticide based on chlorantraniliprole in controlling the codling moth (*C. pomonella*) in apple orchards at the Budisava and Mala Remeta locations during 2023/24, are presented in tables 1-2.

Locality Budisava

At the Budisava locality (2023-24), the number of damaged apples 14 days after the application of the insecticide chlorantraniliprole was significantly lower compared to the control. The insecticide efficacy, at the recommended concentration, was 93.7-95.3%. The number of damaged apples just before harvest was significantly lower in the variant where the insecticide was applied compared to the control. The efficacy was 88.2-90.6% (Table 1).

Table 1. Number of damaged apples and efficacy of the chlorantraniliprole in controlling C. pomonella (Budisava, 2023/24)

Insecticide (%)	2023				2024			
	14 days		22 days		14 days		25 days	
	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%
Chlorantraniliprole (0.02)	0.75±0.9b	95.3	3.5±1.3b	88.2	1.0±0.8b	93.7	2.5±1.3b	90.6
Control	16.0±4.5a	/	29.8±7.9a	/	16±2.2a	/	26.5±4.6a	/
LSD 5%	4.85		6.75		2.03		4.01	
F	21.9		42.7		131.2		97.1	
p	<0.01		<0.01		<0.01		<0.01	

Σ – sum; x–average value; Sd+ -standard deviation; E-efficacy.

Locality Mala Remeta

The efficacy of chlorantraniliprole-based products in controlling the codling moth (*C. pomonella*) in apple orchards at the Mala Remeta locality during 2023/24 is presented in Table 2. The number of damaged apples, 14 days after the application of the insecticide, is significantly lower compared to the control. The efficacy of the tested product was 89.8-92.4%. Just before harvest, the efficacy of the insecticide based on chlorantraniliprole, applied at the recommended concentration, was 89.5-93.0%. The number of damaged fruits in the variants where the tested products were applied is significantly lower compared to the control (Table 2). After the application chlorantraniliprole, no phytotoxic changes were observed on the leaves, shoots, or fruits of the apple varieties Golden Delicious, Idared, and Gala at the Budisava and Mala Remeta locations.

Table 2. Number of damaged apples and efficacy of the chlorantraniliprole in controlling C. pomonella (Mala Remeta, 2023/24)

Insecticide (%)	2023				2024			
	14 days		22 days		14 days		25 days	
	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%
Chlorantraniliprole (0.02)	1.75±0.5b	92.4	1.5±0.6b	93.0	1.2±0.5b	89.8	1.5±0.6b	89.5
Control	23.0±5.9a	/	21.5±2.6a	/	12±4.1a	/	14.2±2.2a	/
LSD 5%	4.85		2.23		3.52		1.90	
F	22.5		218.2		28.2		123.8	
p	<0.01		<0.01		<0.01		<0.01	

Σ – sum; x–average value; Sd+ -standard deviation; E-efficacy.

Based on the studies by Bassi *et al*. (2009), chlorantraniliprole showed a high level of protection for apples against codling moth (*C. pomonella*), with efficacy exceeding 90%. Thanks to its high efficacy, selectivity, and favorable toxicological and ecotoxicological properties, chlorantraniliprole can be significant in anti-resistance strategies as well as in Integrated Pest Management (IPM) programs (Milanese *et al*., 2014). In 2013, the efficacy of insecticides from the avermectin and diamide groups in controlling codling moth (*C. pomonella*) was tested. The results of these trials indicated good efficacy (92.0% and 95.6%) of insecticides from the mentioned chemical groups (Tamaš *et al*., 2018). According to the conducted research by Bosh *et al*. (2018), it was also concluded that chlorantraniliprole showed high efficacy ranging from 85-97% in controlling codling moth, indicating no resistance to chlorantraniliprole, which is consistent with our research. Studies conducted in 2021 in apple orchards at the Budisava location (Vojvodina) showed high efficacy (93.4-94.4%) of chlorantraniliprole-based products in controlling *C. pomonella* (Vuković *et al*., 2022). Perrin *et al*. (2024) tested the effect of temperature (20, 25, 30, and 35 °C) on the toxicity of three insecticides (chlorantraniliprole, emamectin, and spinosad) for *C. pomonella*, as well as their effect on two natural enemies (*Forficula auricularia* L. and *Mastrus ridens* Horstmann) under laboratory conditions. In these tests, chlorantraniliprole showed high efficacy against *C. pomonella* even at high temperatures, while being relatively safe for natural enemies, regardless of temperature or exposure method. This study demonstrated that chlorantraniliprole can be a promising insecticide, especially now that temperatures are rising, as well as for strategies that partially rely on biological control.

CONCLUSIONS

Based on the obtained results, it can be concluded that the codling moth (*Cydia pomonella*) at the tested locations (Budisava, Mala Remeta) showed significant sensitivity to the tested insecticide (chlorantraniliprole). This allows us to continue using the mentioned insecticide in controlling this economically significant apple pest, while adhering to all recommended measures to prevent the development of resistance.

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SUZBIJANJE JABUČNOG SMOTAVCA (*CYDIA POMONELLA* L.) PRIMENOM INSEKTICIDA HLORANTRANILIPROLA

Sažetak

U poslednjih nekoliko decenija, intenzivna proizvodnja jabuke zahteva sve veći nivo tehnologije i znanja kako bi se obezbedio visok prinos i kvalitet proizvoda. U voćnjacima jabuke, pored različitih mera za suzbijanje uključujući agrotehničke i biološke mere zaštite, neophodno je nekoliko puta godišnje primeniti različite pesticidne preparate. Jabučni smotavac je jedna od najvažnijih štetočina u voćnjacima, posebno u jabukama. Često uzrokuje visoke štete, što dovodi do prevremenog opadanja plodova i poteškoća u čuvanju plodova tokom skladištenja, prvenstveno zbog nepravovremene i neadekvatne zaštite. Tokom 2023. i 2024. godine, ogledi su sprovedeni prema standardnim OEPP metodama. Ova studija imala je za cilj procenu efikasnosti sredstva za zaštitu biljaka na bazi hlorantraniliprola (200 g a.s./L, SC), protiv jabučnog smotavca u zaštiti jabuka. U voćnjacima jabuke (sorta Ajdared, Zlatni delišes i Gala) na lokalitetima Budisava i Mala Remeta (Vojvodina, Srbija), preparat na bazi hlorantraniliprola je folijarno primenjen leđnim atomizerom u koncentraciji od 0,02%. Eksperiment je postavljen u četiri ponavljanja u slučajnom blok sistemu. Vizuelnim pregledom registrovano je prisustvo jaja *C. pomonella*, a takođe je ustanovljeno i piljenje gusenica prve (2023) i druge (2024) generacije ove štetočine. Efikasnost testiranog insekticida je procenjena na osnovu broja oštećenih plodova jabuke. Efikasnost je izračunata prema Abbotu, a značaj razlika primenom analize varijanse

(ANOVA) i NZR testa za interval poverenja od 95%. U 2023. godini, četrnaest dana nakon primene, efikasnost hlorantraniliprola iznosila je 95,3% na lokalitetu Budisava, dok je na lokalitetu Mala Remeta efikasnost bila 92,4%. Nakon 22 dana, preparat je pokazao dobru efikasnost koja se kretala od 88,2-93,0%, u zavisnosti od lokaliteta. U 2024. godini, četrnaest dana nakon primene efikasnost je bila 93,7% na lokalitetu Budisava, dok je na lokalitetu Mala Remeta efikasnost bila 89,8%. Nakon 25 dana, insekticid je pokazao dobru efikasnost koja se kretala od 89,5-90,6%, u zavisnosti od lokaliteta. Tokom obe godine, broj oštećenih plodova jabuke u varijantama gde je primenjen hlorantraniliprol bio je značajno niži u odnosu na kontrolu. Na ispitivanim lokalitetima, u obe godine, *C. pomonella* je pokazala značajnu osetljivost na hlorantraniliprol. Ovo ukazuje na to da se ovaj insekticid još uvek može efikasno koristiti za suzbijanje ove ekonomski značajne štetočine u voćnjacima jabuka, pod uslovom da se strogo poštuje antirezistentna strategija.

Ključne riječi: *jabuka, Cydia pomonella, klorantraniliprol, efikasnost*

RASPBERRY LEAF BLOTCH EMARAVIRUS – A SIGNIFICANT THREAT TO RASPBERRY PRODUCTION IN SERBIA*

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Review paper

Abstract

Raspberry leaf blotch emaravirus (RLBV) is a recently characterized virus infecting raspberries. RLBV has been reported in numerous European countries, and it is vectored by the raspberry leaf and bud mite (*Phyllocoptes gracillis*). Virus infection is manifested by light green and yellow leaf blotches and patches, leaf twisting, and distortion of leaf margins. RLBV is the most important virus infecting raspberries in Serbia, causing severe leaf symptoms, including cultivar ‘Willamette’ that is dominant in the orchards, with a share of about 90%.

Results of the conducted research in the last decade confirmed wide distribution and significant genetic diversity of RLBV in Serbia. It was detected in all raspberry-growing regions in all tested cultivars. The fruit of the raspberry ‘Willamette’ proved to be significantly affected by RLBV infection. Depending on the locality and altitude, the recorded reduction of fruit weight was up to 27%. On the other side, soluble solids content in infected fruits was higher. The results of the study on the changes in chemical composition of ‘Willamette’ fruits confirmed the influence of RLBV infection on the examined phenolic profile, but this influence was quite minor compared to the influence of soil composition and weather conditions.

Keywords: *Rubus idaeus L., RLBV, fruit, diversity, yield*

INTRODUCTION

Red raspberry (*Rubus idaeus*) is a host of numerous viruses and virus-like agents (Martin *et al*., 2013). Viral diseases occur in all raspberry-growing regions in the world. Symptoms of viral diseases occur on different plant organs, but the most obvious are on leaves and fruits. The most common symptoms of virus infection on leaves are mottling, chlorosis, yellowing, streak, necrosis, shortening of leaf veins, and scorch. In sensitive cultivars, infected fruits are smaller, deformed, and crumbly. Virus infection can drastically decrease yields and make fruits unmarketable. In some cases, symptoms

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induced by viruses can be mistakenly identified as ones caused by other pathogens or pests.

Figure 1. Severe symptoms of raspberry leaf blotch emaravirus on raspberry leaves

The symptoms of blotches and yellow patches on leaves are frequent occurrences in red raspberry orchards. For a long time, they were described as feeding damage by the raspberry leaf and bud mite (*Phyllocoptes gracillis*) (Dobrivojević and Petanović, 1985; Milenković and Marčić, 2012). While feeding on raspberry plants, raspberry leaf and bud mite causes pale green/yellow patches and blotches, twisting, and distortion of the leaf margins. McGavin *et al*. (2012) detected the virus in raspberry leaf samples with leaf blotch symptoms originating from Great Britain and Serbia. The virus was named raspberry leaf blotch emaravirus (RLBV) (Figure 1). RLBV is a new negative-strand RNA virus that belongs to the genus *Emaravirus*. According to the latest International Committee on Taxonomy of Viruses (ICTV) accepted taxonomy, new species name is *Emaravirus idaeobati* (Kuhn *et al.,* 2023). Its genome is 17,410 nucleotides (nt) long and consists of eight segmented negative sense RNAs (McGavin *et al*., 2012; Lu *et al*., 2015). Each RNA encodes a single open reading frame. RLBV has been confirmed in several European countries: Bulgaria, Bosnia and Herzegovina, Finland, Great Britain, Montenegro, Poland, Serbia, Slovakia, and Ukraine (Pozhylov *et al*., 2021), but also in Kazakhstan (Gritsenko *et al*., 2022). Additionally, there is one unverified report of RLBV in France. RLBV was also detected in the raspberry leaf and bud mite, which indicates its vector role (McGavin *et al*., 2012; Dong *et al*., 2016). RLBV is not included in the EPPO cerification scheme for *Rubus* (Anonymous, 2009) because it was discovered after the last revision of this document. It is still not regulated in any country. Controlling the presence of RLBV is challenging since it is not monitored during the production of raspberry planting material.

RLBV distribution and genetic diversity in Serbia

Raspberry production is an important part of Serbian Agriculture. According to the production quantity and export, Serbia is ranked among the top world countries (Leposavić, 2023). The predominant cultivar is 'Willamette' (90% share), followed by 'Meeker' (5%), 'Tulameen', 'Glen Ample', 'Fertödi Zamatos', 'Polka', 'Himbo Top', 'Enro Sadira' and others. After the discovery of the raspberry leaf blotch emaravirus, the extensive surveys in all raspberry-growing regions of Serbia have been conducted to investigate the presence and distribution of RLBV in the country. RT-PCR with RLBV-specific primers revealed high incidence (68.7%) of RLBV in analysed samples (Jevremović *et al*., 2019). RLBV presence was confirmed in all surveyed locations (Figure 2) in cultivars 'Willamette', 'Meeker', 'Fertödi Zamatos', 'Glen Ample', 'Polana', and 'Tulameen'. Similar research was conducted in Bosnia and Herzegovina by Delić *et al*. (2020). Authors reported RLBV presence in all surveyed locations and cultivars ('Willamette', 'Meeker', 'Fertödi Zamatos', 'Glen Ample', 'Polka', 'Tulameen', and 'Cascade').

The nucleotide sequences of Serbian RLBV isolates showed 93.2−100% identity of the portion of the nucleocapsid (NC) gene (Jevremović *et al*., 2019). Similar results were reported for isolates from Poland (94.6–99.8%) (Cieślińska and Tartanus, 2014), Finland (92.5-100%) (Dong *et al*., 2016), and Bosnia and Herzegovina (92.5-100%) Delić *et al*. (2020).

Figure 2. Map of Serbia showing sampling locations (Jevremović *et al*., 2019)

RLBV impact on the fruit quality and yield

Beside studies on the distribution, prevalence, genetic diversity and phylogeny of the RLBV, important part of the research should be the influence of the plant viruses infection on yield and fruit quality. This type of research has hardly been carried out in fruit tree virology in the last two decades. Researchers are oriented towards research using contemporary molecular techniques to study the genetic diversity, phylogeny, and to discover new viruses using next generation sequencing. There is a lack of research of the economic importance of plant viruses, which is very important for the production and farmers. Bi *et al*. (2012) and Dong *et al*. (2016) stated that RLBV is a cause of significant production losses, but these statements were not supported by research results. The first study on the influence of RLBV on raspberry 'Willamette' fruits, as the most important cultivar in the production in Serbia and surrounding countries, was conducted by Jevremović *et al*. (2022). The study results showed a significant influence of RLBV on fruit dimensions (length, width and height) and weight of infected fruits. The impact of the virus on these fruit traits was clearly evidenced in all four investigated locations in West Serbia, as the most important region for the production of raspberries. The reported decrease in fruit length was 4-9.8%, while fruit width decreased 4.1-9.5%, and fruit height 5.9-14.9% (Figure 3). The decrease in fruit weight in RLBV-infected plants varied from 9.1-27.5%. Fruit chemical properties of 'Willamette' fruits influenced by RLBV infection were also examined by Jevremović *et al*. (2022). Soluble solids contents, titratable acidity, pH value of the fruit juice and total sugars content were evaluated. Soluble solids contents were higher in RLBV-infected fruits and the increase ranged from 1.5-7.4%. No statistically significant effect of RLBV on titratable acidity and pH values of fruits were recorded. The RLBV did not play a significant influence in alterations in total sugar content in infected fruits.

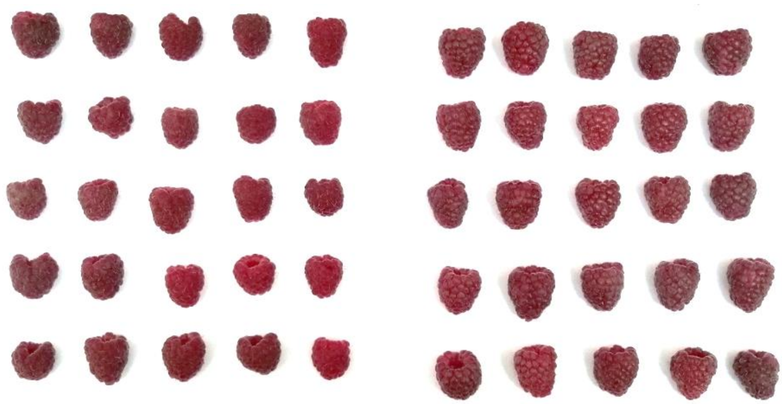

Figure 3. RLBV-infected (left) and healthy fruits (right) (Jevremović *et al*., 2022)

RLBV influence on the polyphenolic profile of fruits

Different factors, as characteristics of the cultivar, maturity stage, agrotechnical treatments, orchard locality, and storage treatment, have an influence on the polyphenolic profile of fruits. Several authors reported the influence of these factors on the phenolic profile of raspberry fruits (Wang *et al*., 2009; Drobek *et al*., 2019; Will *et al*., 2020; Kobori *et al*., 2021). Regarding viruses, as factors that can alterate phenolic profile of raspberries, only one report on the raspberry bushy dwarf virus (RBDV) influence on raspberry 'Meeker' fruits was published (Malowicki *et al*., 2008). Continuing the study on raspberry leaf blotch virus in Serbia, Miletić *et al*. (2024) conducted research to determine the changes in chemical composition of fresh 'Willamette' fruits caused by the RLBV. The trial was set up on three locations during two censecutive years, and included RLBV-infected and RLBV-free plants. This research was oriented to the detection of 11 compounds in raspberry fruit samples: phenolic acids [caffeic acid (CA), p-coumaric acid (pCOU), ferulic acid (FA), ellagicacid (EA)]; flavonol glycosides [quercetin 3-O-rutinoside (rutin, RUT), quercetin 3-Oglucoside (isoquercetin, ISO-Q), quercetin 3-O-rhamnoside (Q3-RHA)]; flavonol aglycones [quercetin (Q), kaempferol (KAE)]; and anthocyanins [cyanidin 3-O-glucoside (chrysanthemin, CY3-GLU), cyanidin 3-O-sophoroside (CY3-SOP)]. RLBV infection showed the least effect on the contents of CA, Q3-RHA, Q, total phenolics (PHENOL), and total anthocyanins (ANTHO) with no statistical significance, while the highest effect was recorded on the contents of pCOU, EA, RUT, ISO-Q, and CY3-SOP. The content of all the detected compounds (except pCOU and Q3-RHA) was significantly influenced by the harvest year and locality. RLBV infection either increased the content of specific polyphenolics (pCOU, EA, RUT, ISO-Q, KAE, CY3-GLU, CY3-SOP) or kept the quantities stable (CA, Q, PHENOL, ANTHO). Only the content of FA reduced following infection.

The study of Miletić *et al*. (2024) confirmed certain susceptibility of 'Willamette' fruits to RLBV infection based on their phenolic composition. Indeed, RLBV infection has a minoir impact on fruit chemical composition compared to soil and environmental conditions.

CONCLUSIONS

Analyzing all the above data, we can conclude that RLBV has become a significant problem in raspberry production in Serbia. Studies demonstrated that RLBV has a considerable impact on fruit quality and yield in red raspberry orchards. The pathogen is not yet regulated and not included in the certification schemes and bypasses control measures. It should be expected that the status of RLBV will soon be regulated and that raspberry planting material will be controlled for its presence.

Acknowledgement

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RASPBERRY LEAF BLOTCH EMARAVIRUS – ZNAČAJNA PRETNJA PROIZVODNJI MALINE U SRBIJI

Sažetak

Virus mrljavosti lista maine (raspberry leaf blotch emaravirus, RLBV) je nedavno okarakterisan virus koji zaražava maline. RLBV je prisutan u brojnim evropskim zemljama, a prenosi ga eriofidna grinja lista maline (*Phillocoptes gracillis*). Simptomi zaraze su svetlozelene i žute mrlje i pege na listovima, uvijanje listova i deformacije ivica lišća. RLBV je najvažniji virus koji zaražava malinu u Srbiji, izaziva jake simptome na lišću različitih sorti, među kojima je i sorta Vilamet koja je dominantno prisutna u zasadima sa učešćem od oko 90%.
Rezultati istraživanja koja su sprovedena u poslednjoj deceniji potvrdili su široku rasprostranjenost i značajan genetski diverzitet RLBV u Srbiji. Prisustvo RLBV je potvrđeno u svim regionima gajenja maline kod svih ispitivanih sorti. Utvrđeno je da RLBV značajno negativno utiče na plodove sorte Vilamet. U zavisnosti od lokaliteta i nadmorske visine zabeleženo je smanjenje mase ploda do 27%. Sa druge strane, sadržaj rastvorljivih suvih materija u zaraženim plodovima bio je veći. Rezultati sprovedenih istraživanja promena hemijskog sastava plodova maline Vilamet pokazali su i uticaj RLBV infekcije na ispitivani fenolni profil, ali je ovaj uticaj bio neznatan u poređenju sa uticajem samog zemljišta i vremenskih uslova.

Ključne reči: *Rubus idaeus L., RLBV, plod, diverzitet, prinos*

GENETIC INSIGHTS INTO APPLE SCAB (*VENTURIA INAEQUALIS* (COOKE) G. WINTER) RESISTANCE IN TRADITIONAL APPLE GERMPLASM OF BOSNIA AND HERZEGOVINA: A GENOME-WIDE ASSOCIATION STUDY

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Original scientific paper

Abstract

A genome-wide association study on resistance to the phytopathogenic fungus *V. inaequalis* was conducted on traditional Bosnian-Herzegovinian apple cultivars. Since the software for conducting GWAS, PLINK ver. 1.9, is adapted to work with diploids, it was necessary to identify and remove triploid varieties beforehand. Triploid identification was performed using axiomFP.py ver. 1.2 software, revealing that 20% of the examined collection were triploids. Based on the ploidy analysis, leaf samples from 35 diploid varieties were collected from the *ex situ* collection in Goražde (Bosnia and Herzegovina) for further research. The disease intensity was then determined by classifying the leaves into six categories (1-6) using the SAD. By calculating McKinney's disease index, a variation was observed, ranging from 27.2% ('Senabija') to 61.1% ('Crvenka'), indicating differences in variety resistance. After isolating and reisolating the pathogen and examining its morphological characteristics, *V. inaequalis* was identified, and its presence in the mentioned orchard was confirmed. GWAS revealed strong signals on chromosomes 04, 05, and 16 for resistance to this pathogen, using high- resolution SNP markers. This study offers insights into the genetic basis of resistance in traditional apple germplasm from Bosnia and Herzegovina to the phytopathogenic fungus *V. inaequalis*, the causal agent of apple scab, highlighting the need for further research to develop varieties that are more resistant to this pathogen.

Keywords: *Malus domestica*, resistance, *V. inaequalis*, SNP markers, GWAS

INTRODUCTION

Apple *(Malus x domestica)* is of significant economic and agronomic importance, as FAOSTAT data indicate that global production reaches 97 million tons annually (FAOSTAT, 2023), making it the third most widely produced fruit in the world, after bananas and watermelons. Traditional apple varieties serve as valuable resources for

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preserving genetic diversity and developing disease-resistant cultivars, as they are more resilient to biotic and abiotic factors, including frost, snow, drought, pests, and pathogens (Zovko *et al*., 2010). To preserve genetic resources, three *ex situ* apple collections have been established in Bosnia and Herzegovina (Srebrenik, Banja Luka, and Goražde). These collections are crucial for conserving genetic diversity and provide a foundation for developing more resistant varieties. Keserović *et al*. (2017) emphasize that these varieties provide essential genes for resistance to diseases like apple scab (*Venturia inaequalis* (Cooke) G.Winter, 1875) and powdery mildew *(Podosphaera leucotrich*a (Ellis & Everh.) E.S. Salmon, 1900). Apple scab, caused by the pathogen *V. inaequalis*, is a significant disease in apple production and one of the most significant in plant pathology (Petreš *et al*., 2022). Modern breeding programs focus on developing disease-resistant cultivars, particularly against apple scab, which causes significant economic losses due to reduced yield and fruit quality, as well as increased fungicide use (Agrios, 2005; Aylor, 1998). The emergence of new *V. inaequalis* strains requires the incorporation of resistance genes, such as the Vf gene (Rvi6) inherited from Malus floribunda 821, into breeding programs for developing new apple varieties (Đorđević *et al*., 2013). Symptoms of this disease include lesions on fruit, leading to reduced yield and quality, with further economic damage potentially caused by secondary pathogens. The disease is particularly problematic in regions with heavy rainfall and requires management to ensure marketable fruit, especially for susceptible apple varieties (Aylor, 1998). The use of single nucleotide polymorphism (SNP) markers is becoming increasingly important for precise genome mapping and identifying genetic variations associated with disease resistance. This study employed a genome-wide association study (GWAS) to identify genomic regions associated with resistance to apple scab using high-resolution markers and bioinformatics tools. This research provides insights into apple scab resistance in traditional apple germplasm of Bosnia and Herzegovina, supporting future breeding efforts to develop more resistant varieties.

MATERIALS AND METHODS

Research location and sampling

Leaf samples of traditional apple varieties were sampled from the *ex situ* apple collection in Goražde (coordinates 43°41'42" N, 19°02'15" E), which includes 55 traditional varieties. No plant protection products were applied in this collection. Leaf samples for the estimation of disease intensity were collected randomly from four sides of the canopy of each diploid apple variety, with four trees per variety. For each of the 35 diploid traditional apple varieties, 30 leaves were collected per cultivar from different parts of the canopy to ensure a representative sample.

SNP data analysis

SNP data for the apple genotypes were obtained using the Axiom® Apple 480 K SNP array (Thermo Fisher Scientific, Waltham, MA, USA) (Bianco *et al*., 2016) previously published in a study by Konjić *et al*. (2023).

Ploidy analysis

Ploidy was determined using axiomFP.py version 1.2 software (Konjić *et al*., 2025). Input files were prepared in Axiom Analysis Suite version 4.0.3 (Affymetrix, Inc.), following the axiomFP.py manual. The analysis was performed in the Terminal with the following command:

```
"python axiomFP.py stat_file.txt ccp_file.txt output".
```

Determining infection intensity

Leaves were classified into six categories (1-6) based on Standard Area Diagram (SAD) (Hasani, 2005), representing disease percentages from 0 to 75% (Table 1). Disease intensity was calculated using the McKinney formula (Numić, 1995), which estimates the percentage of infected tissue area.

Table 1. Leaves classification based on the percentage of the infected leaf area. The percentage interval of leaf infection defines each of the six categories.

Category	***Precentage of infected leaf area (%)***
1	0
2	0.1 - 10
3	10.1 - 25
4	25.1 - 50
5	50.1 - 75
6	> 75

Isolation of *V. inaequalis* fungus

Pathogen isolation was performed according to Agrios (2005) to confirm the presence of infection at the specified site. Isolation was carried out using potato dextrose agar (PDA) as the growth medium. The procedure was conducted under sterile conditions within a laminar flow cabinet to prevent sample contamination. Small sections of infected leaf tissue were surface-sterilized with ethanol and placed onto the nutrient medium in Petri dishes. The plates were incubated in a shaker incubator (ES-20/80, BioSan, Latvia) at 23°C for seven days. Following incubation, re-isolation was performed to obtain pure fungal cultures. Pathogen identification was based on cultural and morphological characteristics (Agrios, 2005).

Quality control of SNP data

Quality control (QC) and pruning of the examined data were performed using PLINK ver. 1.9 software (Purcell et al., 2007) prior to conducting the GWAS. Data quality control was performed using the following command:

```
"./plink bfile 1a --geno 0.2 --mind 0.2 --maf 0.05 out 2a".
```

This command excludes SNPs with more than 20% missing genotypes, as well as those with a minor allele frequency of less than 5%.
Pruning was performed using the following command:

```
"./plink bfile 2a --indep pairwise 50 5 0.5 out 3a".
```

This command retains only those markers that are not in correlation (i.e., not in linkage disequilibrium, LD).

Genome-wide association study

Input files were prepared using Axiom Analysis Suite 4.0.3 (Affymetrix, Inc.). GWAS was performed in PLINK version 1.9 (Purcell et al., 2007) using the "--assoc" command:

```
"./plink --bfile 3a --assoc --out final".
```

Adjusted significance values (Bonferroni correction (Bonferroni), Holm correction (Holm), Sidak Single Step correction SS (Sidak SS), Sidak Step-Down correction (Sidak SD), False Discovery Rate - Benjamini-Hochberg (FDR BH), False Discovery Rate - Benjamini-Yekutieli (FDR BY)) were obtained with:

```
"./ plink--filemydata--assoc--adjust".
```

The p-value distribution was visualized as a Manhattan plot, created using the "qqman" package (Turner, 2018) in R Studio version 4.2.3 (R Studio Team, 2020).

RESULTS AND DISCUSSION

Ploidy analysis

The ploidy analysis using axiomFP.py ver. 1.2 software (Konjić *et al*., 2025) for samples from the *ex situ* collection in Goražde identified 44 out of 55 varieties as diploids and 11 as triploids (Figure 1) (Table 2)

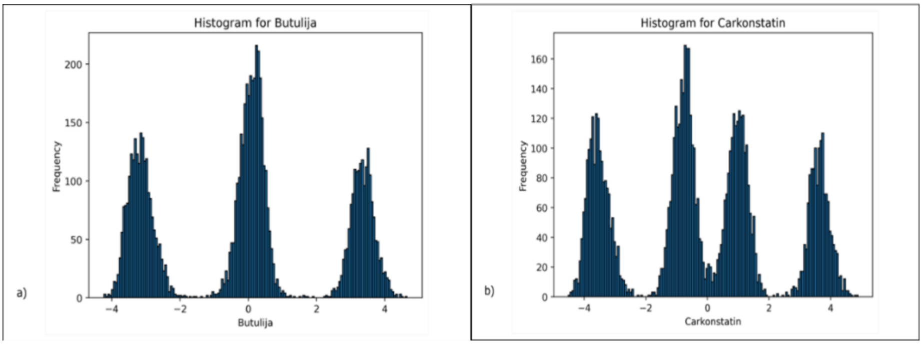

Figure 1. Histograms generated using axiomFP.py ver. 1.2 software:
a) Diploid variety Butulija b) Triploid variety Carkonstantin

Table 2. Ploidy analysis results for all 55 varieties in the *ex situ* collection of Goražde using axiomFP.py software

Nr.	***Variety name:***	***Ploidy:***	***Nr.***	***Variety name:***	***Ploidy:***
1	Amerikanka	2x	30	Švabska zelenika	2x
2	Ananas	2x	31	Zečija glava	2x
3	Babovača	2x	32	Paradija	2x
4	Baščenka	2x	33	Šarenika tvrda	2x
5	Bedrika	2x	34	Sarija	2x
6	Bihorka	2x	35	Bobovec	2x
7	Bistrica	2x	36	Dobrić	2x
8	Bjelka	2x	37	Ruzmarinka	2x
9	Butulija	2x	38	Lijepocvjetka	2x
10	Carkonstatin	3x	39	Lederka	2x
11	Crvenka	2x	40	Budimka	3x
12	Dobrinjka	2x	41	Senabija	2x
13	Dobrinjkina majka	3x	42	Rebrača	2x
14	Kanada	3x	43	Samoniklica	2x
15	Limunka	2x	44	Žuja	3x
16	Ljutika	3x	45	Bobovec	2x
17	Lorinka	3x	46	Petrovača bijela	2x
18	Misirka	2x	47	Tetovka	3x
19	Muslimača	2x	48	Prijedorska zelenika	3x
20	Ovčiji nos	2x	49	Pamuklija	2x
21	Pazarka	2x	50	Bukovija	2x
22	Petrovača crvena	2x	51	Habikuša	2x

23	Posavka	2x	52	Kanjiška	3x
24	Prisatka	2x	53	Srebrenička	2x
25	Ranka	2x	54	Funtača	3x
26	Šimširka	2x	55	Đulabija	2x
27	Srčika	2x			
28	Srebrenjača	2x			
29	Sulija	2x			

Previous studies using SSR markers showed that 27% of apple genotypes in Bosnia and Herzegovina are triploid (Gaši *et al.,* 2013), based on an analysis of 51 apple varieties from the *ex situ* collection in Srebrenik, including both local and reference varieties. Similar studies report 28% triploids in northwestern Spain (Pereira-Lorenzo *et al*., 2007) and 12% in Norway (Gaši *et al*., 2016). These results suggest that triploidy is more characteristic of apple varieties originating from the Balkans, reflecting a higher frequency of triploids among genetic resources from southern Europe compared to those from northern Europe.

Determination of infection intensity

The results of infection intensity assessment for the evaluated diploid apple varieties are presented in Table 3.

Table 3. Disease index for 35 apple varieties

Nr.	*Variety name:*	*Disease Index (%)*	*Nr.*	*Variety name:*	*Disease Index (%)*
1	Butulija	40	19	Paradija	35
2	Bihorka	51.6	20	Pamuklija	48.3
3	Bukovija	33.8	21	Pazarka	40
4	Baščenka	27.7	22	Prisatka	35
5	Babovača	47.2	23	Posavka	46.6
6	Bedrika	40	24	Petrovača crvena	34.4
7	Bobovec	36.1	25	Ranka	31.6
8	Bistrica	32.7	26	Ruzmarinka	50.5
9	Bjelka	36.1	27	Srčika	38.3
10	Crvenka	61.1	28	Samoniklica	33.8
11	Dobrić	42.7	29	Sulija	28.3
12	Đulabija	48.8	30	Senabija	27.2
13	Dobrinjka	42.2	31	Srebrenička	37.2
14	Habikuša	27.7	32	Šarenika tvrda	32.2

15	Limunka	36.6	33	Švabska zelenika	33.3
16	Lijepocvjetka	29.4	34	Šimširka	45
17	Muslimača	37.7	35	Zečija glava	40.4
18	Ovčiji nos	36.6			
Average rate: 38.4%					

McKinney's disease index ranged from 27.2% ('Senabija') to 61.1% ('Crvenka'), showing variation in resistance among varieties. The average disease index for all analyzed varieties was 38.4%, reflecting their susceptibility to the disease. Bignami et al. (2001) found high variation in resistance to *V. inaequalis* among 50 Italian apple varieties, with older varieties being less susceptible than modern ones, such as 'Golden Delicious'. Differences in disease indices are influenced by various factors, including climate, soil, genetics, and regional pathogen strains.

Isolation and identification of pathogens

Leaves with dark green to brownish-green spots were brought to the lab for pathogen isolation on PDA medium, re-isolation for pure culture (Figure 2), and microscopic examination. Results were compared with scientific references (MacHardy, 1996), confirming the presence of *V. inaequalis* in the *ex situ* collection orchard.

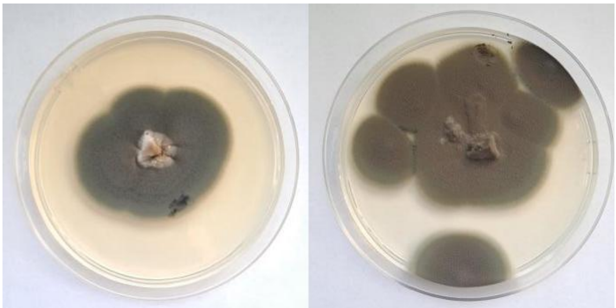

Figure 2. The isolated *V. inaequalis* on PDA

Genome-Wide Association Study

The genome-wide association study revealed strong association signals on chromosomes 04, 05, and 16 for resistance to the *V. inaequalis* pathogen (Figure 3). The reference genome '*Malus x domestica GDDH13 v1.1*' (Daccord *et al.,* 2017) was used for identifying SNP positions. Notably, the SNP 'AX-115221833' located on chromosome 5 (05) showed the most significant association, with a p-value of 0.000001252, even after applying correction factors, including the Bonferroni, Holm, and Sidak tests

Several SNPs on chromosome 16 (16), including 'AX-115546257', 'AX-115551791', 'AX-115571801', 'AX-115599030', 'AX-115614189', 'AX-115615770', and 'AX-115476689', also showed significant associations with p-values of 0.000007452. Finally, the SNP 'AX-115290446' on chromosome 4 (04) had a p-value of 0.000008483, with a slightly lower level of significance (Appendix 1).

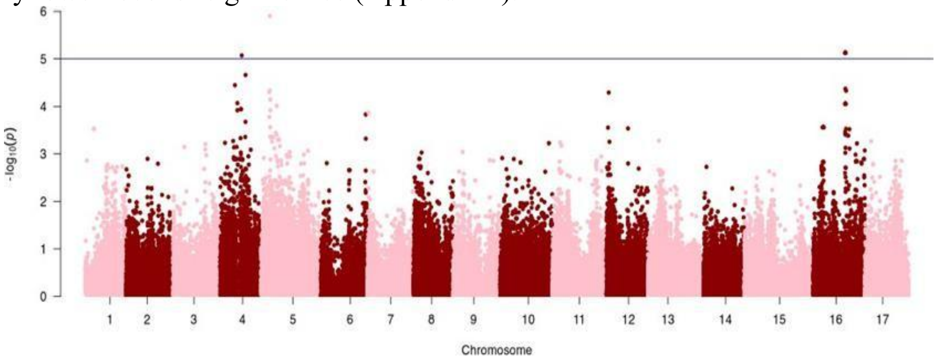

Figure 3. Manhattan plot of the GWAS results, showing strong association signals on chromosomes 4, 5, and 16.

McClure *et al*. (2018) identified strong association signals for resistance to apple scab, caused by the fungus *V. inaequalis*, on chromosomes 01, 04, 07, 09, 10, 12, and 15 using GBS (Genotyping-by-Sequencing). Both studies found significant associations on chromosome 4, suggesting this region is important for resistance to *V. inaequalis* regardless of differences in location and methodology.

CONCLUSIONS

This study demonstrated that traditional apple cultivars from Bosnia and Herzegovina exhibit significant variability in their resistance to *V. inaequalis,* underscoring their value as genetic resources for developing new scab-resistant varieties.
The research led to the following conclusions:

- axiomFP.py version 1.2 identified 20% triploid genotypes in the collection, which is lower than in northwest Spain but similar to results from other studies in the Balkan region.
- Analysis of disease intensity showed significant variation in resistance among traditional apple varieties, with an average disease index of 38.4%. 'Senabija' was the most resistant (27.2%), while 'Crvenka' had the highest disease index (61.1%). The variations in resistance highlight the importance of preserving genetic diversity and utilizing traditional varieties in disease control strategies.
- After isolation, re-isolation, and microscopic analysis, the presence of the pathogen *V. inaequalis* was confirmed in the *ex situ* orchard in Goražde.
- The genome-wide association study identified strong association signals on chromosomes 04, 05, and 16 through SNP markers, which are linked to

- resistance to the disease caused by the *V. inaequalis* pathogen. This research enhances our understanding of the genetic basis of resistance and opens up possibilities for marker-assisted selection (MAS). Notably, the SNP marker on chromosome 5 showed the strongest association with the resistance trait.

The collected data contribute to the conservation of local apple germplasm and highlight the need for its systematic integration into modern breeding programs. Future research should focus on expanding the range of tested genetic material, functionally validating the identified loci, and assessing the stability of resistance under various agroecological conditions. The findings are not only scientifically significant but also provide practical guidelines for improving sustainable fruit production in Bosnia and Herzegovina. This approach aims to reduce the reliance on plant protection products and enhance the resilience of local agriculture against the challenges posed by climate change.

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ISPITIVANJE GENOMSKIH ASOCIJACIJA OTPORNOSTI NA FUZIKLADIJ (*VENTURIA INAEQUALIS* (COOKE) G. WINTER) KOD TRADICIONALNIH BOSANSKOHERCEGOVAČKIH KULTIVARA JABUKE

Sažetak

Ispitivanje genomskih asocijacija otpornosti na fitopatogenu gljivu *V. inaequalis* provedeno je na lokalitetu Goražde, fokusirajući se na tradicionalne bosanskohercegovačke kultivare jabuke. Budući da je softver za provođenje analize genomskih asocijacija (engl. *Genome wide association studies,* GWAS), PLINK ver. 1.9 prilagođen za rad s diploidima, bilo je neophodno identificirati i ukloniti triploidne sorte prije same analize. Identifikacija triploida izvršena je upotrebom FP.py ver. 1.2 softvera, pri čemu je identificirano da je ukupan postotak triploidnosti u ispitivanoj kolekciji iznosio 20%. Na temelju rezultata provedene analize ploidnosti, iz *ex situ* kolekcije u Goraždu prikupljeni su uzorci listova od 35 diploidnih sorti za daljnje istraživanje. Nakon toga pristupilo se određivanju intenziteta oboljenja. Listovi su klasificirani u šest kategorija (1-6) zasnovanih na Standard Area Diagramu (SAD). Izračunavanjem McKinney-evog indeksa oboljenja, uočena je varijacija od 27,2% ('Senabija') do 61,1% ('Crvenka'), ukazujući na razlike u otpornosti sorti. Nakon izolacije i reizolacije patogena, te mikroskopiranja morfoloških karakteristika patogena, identificiran je patogen *V. inaequalis* kao i prisustvo patogena u navedenom voćnjaku. Analiza genomskih asocijacija otkrila je snažne signale na hromosomima Chr04, Chr05 i Chr16 za otpornost na ovog patogena, koristeći markere visoke rezolucije jednonukleotidnih polimorfizama (engl. *Single nucleotide polymorphisms*, SNP). Ova studija pruža uvide u genetsku osnovu otpornosti tradicionalnih bosankohercegovačkih kultivara jabuke na fitopatogenu gljivu *V. inaequalis*, uzročnika oboljenja čađave krastavosti, naglašavajući potrebu za daljnjim istraživanjem radi razvoja sorti koje su otpornije na pomenutog patogena.

Ključne riječi: *Malus domestica*, *otpornost, V. inaequalis, SNP markeri, GWAS*

APPENDIX

Appendix 1. Table showing the first 17 out of 125113 rows of the GWAS results. **BP** *(Base Pair position)* - **SNP** position on the chromosome; **SNP** - identifier for the SNP; **UNADJ** (*Unadjusted p-value*) - raw p - value for the correlation between SNP and trait of interest, without any correction for multiple testing; **GC** *(Genetic Clusters*) – p - value after correction for population stratification using genomic control; **BONF**; **HOLM**; **SIDAK SS**; **SIDAK SD***;* **FDR BH**; **FDR BY**.

	BP	SNP	UNADJ	GC	BONF	HOLM	SIDAK SS	SIDAK SD	FDRBH	FDR BY	CHR
1	7320635	AX-115221833	0.000001252	0.000001252	0.1617	0.1617	0.1493	0.1493	0.1204	1.0000	5.0000
2	25587562	AX-115546257	0.000007452	0.000007452	0.9628	0.9628	0.6182	0.6182	0.1204	1.0000	16.000
3	25581299	AX-115614189	0.000007452	0.000007452	0.9628	0.9628	0.6182	0.6182	0.1204	1.0000	16.000
4	25680916	AX-115615770	0.000007452	0.000007452	0.9628	0.9628	0.6182	0.6182	0.1204	1.0000	16.000
5	25621939	AX-115476689	0.000007452	0.000007452	0.9628	0.9628	0.6182	0.6182	0.1204	1.0000	16.000
6	25849373	AX-115551791	0.000007452	0.000007452	0.9628	0.9628	0.6182	0.6182	0.1204	1.0000	16.000
7	25642249	AX-115599030	0.000007452	0.000007452	0.9628	0.9628	0.6182	0.6182	0.1204	1.0000	16.000
8	25572197	AX-115571801	0.000007452	0.000007452	0.9628	0.9628	0.6182	0.6182	0.1204	1.0000	16.000
9	17113966	AX-115290446	0.000008483	0.000008483	1.0000	1.0000	0.6658	0.6658	0.1218	1.0000	4.0000
10	20182320	AX-115617616	0.000021850	0.000021850	1.0000	1.0000	0.9406	0.9406	0.2567	1.0000	4.0000
11	11687020	AX-115618330	0.000035800	0.000035800	1.0000	1.0000	0.9902	0.9902	0.3854	1.0000	4.0000
12	25715508	AX-115514222	0.000042600	0.000042600	1.0000	1.0000	0.9959	0.9959	0.3890	1.0000	16.000
13	7347877	AX-115344506	0.000046490	0.000046490	1.0000	1.0000	0.9975	0.9975	0.3890	1.0000	5.0000
14	26252120	AX-115428382	0.000046770	0.000046770	1.0000	1.0000	0.9976	0.9976	0.3890	1.0000	16.000
15	6753434	AX-115478589	0.000049690	0.000051180	1.0000	1.0000	0.9984	0.9984	0.3890	1.0000	5.0000
16	1797312	AX-115383236	0.000051180	0.000049690	1.0000	1.0000	0.9987	0.9987	0.3890	1.0000	12.000
17	7689478	AX-115581359	0.000071770	0.000071770	1.0000	1.0000	0.9999	0.9999	0.4561	1.0000	5.0000

ASSESSING PESTICIDE RISK IN CERTAIN FRUITS AND VEGETABLES: A COMPREHENSIVE EVALUATION*

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Original scientific paper

Abstract

Climate changes, freshwater scarcity, diminishing arable land, and particularly the prevalence of harmful agents pose significant challenges to food production, necessitating robust plant protection measures. Contemporary science faces several key issues, including increasing concerns regarding food safety, the rising trend of organic farming, the emergence of resistant populations of pests, and the loss of biodiversity due to chemical pesticide use. It is therefore crucial to protect consumers from exposure to unacceptable levels of pesticides through ongoing monitoring programs. Over the past decade, the presence of food containing pesticide residues has risen significantly, raising major safety concerns for consumers globally. Consequently, the detection and quantification of these residues in food have become paramount. Therefore, evaluation the adverse health effects of pesticides through risk assessment are still required. This is particularly important for food mainly consumed fresh, such as fruits and vegetables. This study assessed the health risks associated with pesticide residues from the classes of diamides, spinosyns, strobilurins, and methoxycarbamates in various fruits and vegetables. The evaluation involved calculating the Estimated Daily Intake (EDI), Hazard Quotient (HQ), and Hazard Index (HI). The research was conducted based on findings from a dissipation experiment. Results indicated that the detected residue levels fell within safe limits, suggesting that their consumption does not present a significant health risk to humans.

Keywords: *pesticide residues, risk assessment, fruits, vegetables*

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INTRODUCTION

During the historical development of plant protection, the number of introduced pesticides effective in controlling numerous harmful organisms grew. Therefore, they have helped enhance the quality and quantity of yields globally (Damalas and Eleftherohorinos, 2011). However, during the long-term intensive application of pesticides, negative effects were manifested in many fields. Due to their ability to retain and accumulate in the environment, and food, growing concerns are focused toward these negative effects (Akoto *et al*., 2013). Although the development of new pesticide substances and their further application are legally regulated, there are ongoing problems related to their residues, causing further harmful effects (Damalas, 2009). To address these challenges, modern agricultural practices involve the application of pesticides, i.e. plant protection products (PPP), with more favorable ecotoxicological characteristics and a reduced pre-harvest interval (PHI) (Lazić *et al*., 2012).

Food is essential for human health and represents the primary pathway of human intake of pesticides (Juraske *et al*., 2009). Therefore, evaluating pesticides' potential health risks remains indispensable, particularly for freshly consumed food, such as fruit and vegetables.

The dietary intake of pesticide residues can be assessed with varying levels of precision, ranging from estimates based on the theoretical maximum daily intake (TMDI) to more accurate assessments using the estimated daily intake (EDI) (UNEP/FAO/WHO, 1989). The detection and quantification of pesticide residues in food is crucial for assessing the extent of human exposure to these substances. Pesticide residues in food are typically monitored regarding Maximum Residue Level (MRL) (Akoto *et al*., 2013). Health risks associated with those residues, especially in fruits and vegetables, can be evaluated through methods such as calculating the Estimated Daily Intake (EDI), Hazard Quotient (HQ), and Hazard Index (HI). Risk assessment involves comparing potential long- and short-term dietary pesticide intake with two key hazard characterization parameters: Acceptable Daily Intake (ADI) and Acute Reference Dose (ARfD). Short-term dietary exposure is evaluated by comparing the International Estimated Short-Term Intake (IESTI) with the ARfD. This calculation is derived from data gathered in field trials offering a reliable evaluation of acute health risks (Struciński *et al*., 2006). Long-term dietary exposure is assessed by comparing the Theoretical Maximum Daily Intake (TMDI) with the ADI. The TMDI assumes that food consumed over a lifetime contains pesticide residues at the MRL level, and is calculated using MRL values and the average daily consumption of food for which MRLs are established (Osman, 2011). In contrast, evaluating the Hazard Risk (HR) through EDI incorporates median residue values from trials, considering additional factors for more precise estimation.

This study assessed the health risks associated with residues of pesticides belonging to the chemical groups of diamides, spinosyns, strobilurins, and methoxy-carbamates in various fruits and vegetables. Strobilurins are fungicides widely used in agriculture due to their effectiveness in controlling fungal diseases, acting preventively and systemically. Methoxycarbamates, characterized by a methoxy group in their structure,

are usually applied as insecticides, fungicides, and herbicides, demonstrating efficacy. Diamides, which contain two amide linkages in a single molecule, are utilized for crop protection against insects, fungi, and certain weeds. Spinosyns, natural insecticides derived from the fermentation of *Saccharopolyspora spinosa*, are effective in controlling a broad spectrum of insect pests, with low toxicity to non-target organisms, making them commonly used in ecological farming systems.
All these pesticide groups play a significant role in plant protection within conventional agriculture, however, their application requires careful management to reduce the risk of resistance and negative environmental and health effects.

MATERIALS AND METHODS

In order to obtain data for the calculation of dietary risk assessment, field experiments were conducted at three localities (Kula, Mala Remeta, Gospođinci) in the Republic of Serbia. In this study, diamides insecticides chlorantraniliprol, cyantraniliprol, spinosynes insecticide spinetoram, strobilurine fungicide boscalid and methoxycarbamates fungicide pyraclostrobin were used. They are applied according to EPPO methods, during ripening stage, at the recomended rate for the control of economicaly important pests (Table 1).
Insecticides chlorantraniliprol and cyantraniliprol were applied in peach, for the control of *Cydia molesta* and *Trialeurodes vaporariorum* in tomato, spinetoram was used for the control of *Psylla piri* in pear, while fungicides boscalid and pyraclostrobin were applied in strawberry and raspberry against *Botrytis cinerea*.

Table 1. Pesticides used in the study

Active substance	The amount of a.s. in PPP	Recommended application rate	Fruit/ vegetable	BBCH stage	PHI (days)	MRL (mg/kg)
chlorantraniliprol	100 g/l	1.2 l/ha	peach	74	14	1
cyantraniliprol	100 g/l	0.6 l/ha	peach tomato	74 74	7 1	1.5 0.6
spinetoram	200 g/kg	0.3 l/ha	pear	75	7	0.15
boscalid pyraclostrobin	267 g/kg 67 g/kg	1.5 l/ha	strawberry	65-87	7	6 1.5
boscalid pyraclostrobin	267 g/kg 67 g/kg	1.5 l/ha	raspberry	65-87	7	10 1.5

Sampling was performed 1 hour after application (after drying depozit), and afterward daily during pre-harvest interval. Pesticide resiudes were determined by previously validated the QuEChERS-based method followed with HPLC.

HQ value was calculated by dividing EDI (mg/kg bw/day) with the relevant ADI (mg/kg bw/day) value (Łozowicka *et al*., 2013), while average consumption and acceptable daily are taken from the EFSA. EDI was calculated using data for the concentration of pesticide obtained in the field experiment, food consumption, and body weight.

RESULTS AND DISCUSSION

Risk assessment was performed based on the results of the field experiments (Žunić *et al*., 2020; Lazić *et al*., 2020; Šunjka *et al*., 2021). For the analysis, only the highest residue level of the insecticides and fungicides in samples, obtained in the dissipation studies, were taken. Samples were collected 1 hour after the application, representing the samples with the highest risk for the consumers.

For long-term risk assessment, HQ was evaluated by comparing the exposure with the toxicological reference value (ADI) (Sharma *et al*., 2022). The EDI values were calculated for analyzed fruits and vegetable samples and the results obtained are summarized in Table 2.

None of the fruit and vegetable samples reported HQ values >1 thereby indicating that an unacceptable risk of daily intake was not observed in these experiments. Values of HQ between 0.1 and 1.0 were obtained for cyantraniliprole in peaches (0.14931) and tomatoes (0.60407), indicating a moderate risk of cyantraniliprole in this fruit and vegetable. A hazard quotient below 0.1 was in the analysis of spinetoram in pear, as well as boscalid and pyraclostrobin in strawberry and raspberry, with a low risk of their use.

Mainly, there is a lack of data on pesticide risk assessment in fruits and vegetables. Studies of risk assessment of chlorantraniliprole and cyantraniliprole in tomato and paddy ecosystems showed a moderate risk for these insecticides (Paramasivam *et al*., 2021; Mahato *et al*., 2023).

Table 2. Results of the evaluation of risk assessment

		ACA/day (kg/day)	Average adults body weight (kg)	Residue level (mg/kg)	EDI (mg/kg/ bw/ day)	ADI (mg/kg/ bw/day)	Hazard quotient (HQ)
pear	spinetoram	0.0074	70.8	0.51	0.00005	0.024	0.00222
peach	cyantraniliprole	0.0167	70.8	6.33	0.00149	0.01	0.14931
peach	chlorantraniliprole	0.0167	70.8	10.14	0.00239	1.56	0.00153
strawberry	boscalid	0.0044	70.8	12.10	0.00075	0.04	0.01880
	pyraclostrobin	0.0044	70.8	10.93	0.00068	0.04	0.01698
raspberry	boscalid	0.0027	70.8	17.6	0.00067	0.04	0.01678
	pyraclostrobin	0.0027	70.8	9.25	0.00035	0.04	0.00882
tomato	cyantraniliprole	0.0990	70.8	4.32	0.00604	0.01	0.60407

ACA – average consumption of adults

CONCLUSIONS

In this study, the maximum residue levels of pesticides obtained one hour after the application were used to evaluate risk assessment as the worst-case scenario. The results indicate that their consumption does not present a significant health risk to humans, however, prescribed PHI values have to be considered.

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PROCENA RIZIKA OD PESTICIDA U ODREĐENIM VRSTAMA VOĆA I POVRĆA: SVEOBUHVATNA EVALUACIJA

Sažetak

Klimatske promene, nedostatak vode, smanjenje obradivog zemljišta, a posebno rasprostranjenost štetnih agenasa, predstavljaju značajne izazove u proizvodnji hrane, što zahteva adekvatne mere zaštite bilja. Istovreemeno, savremena nauka se suočava sa nekoliko ključnih pitanja kao što je rastuća zabrinutost za bezbednost hrane, trend organske poljoprivrede, pojava rezistentnih populacija štetetnih organizama i narušavanje biodiverziteta, usled upotrebe hemijskih pesticida. Stoga je jedna od ključnih mera zaštita potrošača od izloženosti neprihvatljivim nivoima pesticida. Tokom protekle decenije, prisustvo hrane koja sadrži ostatke pesticida značajno je poraslo. Shodno tome, neophodna je kontinuirana provera prisustva ostataka pesticida u poljoprivrednim proizvodima, a posebno u hrani koja se uglavnom konzumira u svežem stanju, kao što su voće i povrće. Ovim istraživanjem je, na osnovu procenjenog dnevnog unosa (EDI) i količnika opasnosti (HQ), izvršena procena rizika od ostataka pesticida iz klase diamida, spinozina, strobilurina i metoksikarbamata u različitom voću i povrću. Procena je uključivala izračunavanje bazirano na eksperimentima dinamike degradacije, a rezultati su pokazali da ostaci analiziranih pesticida u uzorcima voća i povrća ne predstavlja značajan rizik po zdravlje ljudi.

Ključne reči: *ostaci pesticida, procena rizika, voće, povrće*

HEALTH STATUS AND ITS EFFECTS ON DAILY MILK PRODUCTION IN SIMMENTAL COWS CONSIDERING KEY ANIMAL-RELATED FACTORS*

Boris Ljubojević[1], Zvonimir Steiner[2], Ranko Gantner[2], Klemen Potočnik[3], Vesna Gantner*[2]

Original scientific paper

Abstract

This study analyzed the effect of mastitis on daily milk production parameters in Simmental cows, considering factors such as lactation stage, parity, and production level. After applying quality control measures, the final dataset consisted of 2,835,101 test-day records of dairy Simmental cows. The results showed that both subclinical and clinical mastitis negatively impacted milk yield and milk composition. Cows with subclinical mastitis had lower milk production compared to healthy cows, while those with clinical mastitis experienced even more significant losses. Mastitis also influenced milk quality, with increased fat and protein content and decreased lactose levels. The effects were more pronounced in cows in later stages of lactation and those with higher production levels. Early detection and intervention are critical to minimize milk production losses and maintain milk quality. Improving farm management practices, such as monitoring somatic cell counts and ensuring proper hygiene and nutrition, is essential for optimizing milk production and reducing economic losses.

Key words: *mastitis, dairy Simmental, milk production, somatic cell count*

INTRODUCTION

Production diseases in dairy cows are primarily caused by management practices, especially poor feeding and handling. These diseases encompass metabolic disorders, infertility, mastitis, and laminitis, with nutritional and managerial factors being pivotal (Nir, 2003). Although infectious diseases often garner more public attention, production diseases have a far greater economic impact, which surpasses that of epidemic outbreaks (Hogeveen *et al.*, 2019). Addressing these concerns through better management practices not only improves animal health but also enhances the economic efficiency of dairy production. Mastitis, the most common production disease in dairy cattle (Seegers *et al.*, 2003), is primarily caused by intramammary infections (IMI) from bacterial

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pathogens. It results in physical, chemical, and bacteriological changes in milk, as well as pathological alterations in glandular tissue. Mastitis can be classified into clinical and subclinical forms. Clinical mastitis presents with visible symptoms, such as abnormal milk, gland inflammation, and systemic illness, whereas subclinical mastitis is asymptomatic and is identified by elevated somatic cell counts (SCC) (Adkins & Middleton, 2018). Clinical mastitis is further classified based on severity: mild (milk abnormalities), moderate (gland inflammation), and severe (systemic symptoms) (Narváez-Semanate *et al.,* 2022). Mild cases are the most common, while severe cases are relatively rare. Additionally, mastitis has a significant impact on milk production, quality, and farm economics, underscoring the importance of early detection and intervention. Mastitis diagnosis is based on clinical observations and measurements of the inflammatory response, with the identification of the causative agent being essential for diagnosing intramammary infections (IMI). Subclinical mastitis, which is more common than clinical forms, is responsible for up to 80% of milk production losses and represents a significant long-term risk (Halasa *et al*., 2007). If left untreated, affected udder quarters may become nonfunctional, leading to culling or even death. Despite its economic impact, preventive measures are often delayed until a decrease in milk yield is observed. Early detection is crucial, as changes in udder tissue occur before visible symptoms (Argaw, 2016). Prompt diagnosis can help reduce antibiotic use, maintain milk production, and prevent the spread of infection (Kamal *et al.,* 2014). Additionally, mastitis contributes to greenhouse gas emissions, highlighting the importance of prevention for both sustainability and profitability (Özkan Gülzari *et al*., 2018). Effective management not only minimizes losses and optimizes culling rates but also reduces emissions per kilogram of milk produced.

Somatic cell count (SCC) is a vital indicator of intramammary infections and milk quality, monitored at the individual, herd, and population levels (Schukken *et al*., 2003). Monitoring SCC offers valuable insights into udder health, as elevated counts usually signal inflammation caused by pathogen invasion, which triggers an immune response and leads to an increase in somatic cells in the milk (Ivanov *et al*., 2016; Alhussien & Dang, 2018). An increase in SCC across successive lactations is mainly associated with polymorphonuclear leukocytes (PMN), while fluctuations in SCC within a lactation involve both PMN and other immune cells (Blackburn, 1966). Various factors influence SCC variability, including lactation stage, parity, season, management practices, and environmental conditions. SCC tends to rise with lactation number due to cumulative udder stress and immune system changes (Mikó *et al.,* 2016). Cows in late lactation often show higher SCC levels due to prolonged milk stasis and diminished immune function. Heat stress further aggravates SCC fluctuations, as demonstrated in studies on Holstein cows, where SCC levels varied according to temperature-humidity index, milk yield, breed, and parity (Gantner *et al.,* 2011, 2017). Moreover, herd management plays a critical role in controlling SCC, as poor milking hygiene, improper equipment maintenance, and inadequate housing conditions contribute to bacterial infections and increased SCC (Hadrich *et al*., 2018). Healthy udder function is typically indicated by SCC levels below 100,000 cells/ml, with optimal milk quality falling within the range of 20,000 to 100,000 SCC/ml (Mikó *et al*., 2016). Elevated SCC is associated with considerable production losses, with cows exhibiting SCC levels between 50,000 and 100,000 cells/ml experiencing a loss of more than 8%, and those with SCC levels ranging from 100,000 to 250,000 cells/ml suffering losses of 15–18% (Pfützner & Ózsvári, 2016). The overall impact on milk yield is influenced by the distribution of SCC across cows and the parity within the herd (Chen *et al*., 2021). Therefore, managing SCC through improved hygiene, nutrition, and environmental control is crucial for maintaining milk production efficiency and minimizing economic losses.

Given the significance of mastitis analysis on dairy cattle farms, this study aimed to examine the effect of animal health status (normal, subclinical, or clinical mastitis) on daily milk production parameters in Simental cattle, taking into account key animal-related factors such as lactation stage, parity, and production level.

MATERIALS AND METHODS

The statistical analysis in this study was based on test-day records from Simental cows, collected over a 10-year period (January 2013–December 2022) as part of routine milk recording. The milk recording process followed the standards set by the International Committee for Animal Recording (ICAR, 2017) for alternative milk recording methods (AT4/BT4). Under this system, milk yield measurements and sample collection were performed every four weeks, with samples taken from each cow during either morning or evening milking. Data collection for the AT4 method was conducted by trained officers from the Croatian Agency for Agriculture and Food (HAPIH), while farm employees trained in milk recording were responsible for the BT4 method. After collection, milk samples were transported to the accredited Central Laboratory for Milk Quality Control (SLKM) of HAPIH for analysis, in full compliance with ICAR (2017) protocols.
Milk composition analysis included parameters such as fat, protein, lactose, total solids, solids-not-fat, urea, casein, free fatty acids, pH, ketone bodies, and freezing point. These parameters were determined using infrared spectrophotometry on MilkoScan analyzers. Somatic cell counts were measured using Fossomatic analyzers, utilizing the fluoro-opto-electronic method.Materials and Methods Materials and Methods Materials and Methods Materials and Methods Materials and Methods Materials and Methods Materials and Methods Materials and Methods Materials and Methods Materials and Methods Materials and Methods Materials and Methods Materials and Methods
Before implementing logical data control procedures, the dataset obtained from HAPIH contained over 4 million test-day records. During the validation process, entries that did not meet the standards set by ICAR (2017) were excluded. Additionally, records with missing or inaccurate information regarding breed, farm identification, breeding region, lactation stage, parity, age at first calving, calving date, and milk recording date were

removed to ensure data integrity and consistency. After applying these quality control measures, the final dataset consisted of 2,835,101 test-day records from Simental cows. Somatic cell count (SCC) served as the primary indicator for identifying subclinical and clinical mastitis. Based on SCC levels, cows were categorized into three groups: healthy (< 200,000 cells/ml), subclinical mastitis (200,000–400,000 cells/ml), and clinical mastitis (> 400,000 cells/ml). To account for the effect of lactation stage, cows were divided into four days-in-milk (DIM) classes: < 100 days, 100–200 days, 200–300 days, and > 300 days. Additionally, cows were grouped according to parity into four categories: first (I), second (II), third (III), and fourth or higher (≥ IV). Cows were also categorized by their daily milk yield (DMY) into four production groups: I. DMY < 20 kg, II. DMY between 20 and 30 kg, III. DMY between 30 and 40 kg, and IV. DMY > 40 kg.

The analysis of the effect of animal health status (normal, subclinical, or clinical mastitis) on daily milk production parameters in Simental cattle was conducted separately for the classes of lactation stage, parity, and animal production level. The statistical model used for the analysis is as follows:

$$y_{ijklmn} = \mu + b_1\left(\frac{d_i}{305}\right) + b_2\left(\frac{d_i}{305}\right)^2 + b_3\,ln\left(\frac{305}{d_i}\right) + b_4\,ln^2\left(\frac{305}{d_i}\right)$$

$$+A_j + R_k + H_l + S_m + M_n + e_{ijklmn}$$

where:

y_{ijklmn} = estimated milk production trait (daily milk yield, daily fat, protein, lactose and urea content, log value of somatic cell count);

μ = intercept;

b_1, b_2, b_3, b_4 = regression coefficients;

d_i = stage of lactation (i = 6 to 400 day);

A_j = fixed effect of age at first calving (j = 21 to 36 month) * only for firs parity;

R_k = fixed effect of region k (k = Central, Eastern, Mediterranean);

H_l = fixed effect of herd size (l = I, II, III, IV, V, VI);

S_m = fixed effect of season (m = spring, summer, autumn, winter);

M_n = fixed effect of animal health status M (n = healthy, subclinical mastitis, and clinical mastitis);

e_{ijklmn} = residual.

The significance of the differences between the estimated LSMeans was tested by Scheffe's method of multiple comparisons using the MIXED procedure of SAS (SAS Institute Inc., 2019)

RESULTS AND DISCUSSION

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Table 1. LsMeans of daily milk production parameters regarding the Simentals health status and considering lactation stage classes (< 100 days, 100 – 200 days, 200 – 300 days, > 300 days)

Lactation stage class	Health status	DMY	DFC	DPC	DLC	SCClog	UREA
< 100 days	Normal	$17,98^{A}$	$3,92^{A}$	$3,20^{A}$	$4,56^{A}$	$5,51^{A}$	$19,45^{A}$
	Subclinical	$17,68^{B}$	$4,01^{B}$	$3,27^{B}$	$4,48^{B}$	$8,10^{B}$	$18,97^{B}$
	Clinical	$17,53^{C}$	$4,06^{C}$	$3,30^{C}$	$4,38^{C}$	$10,03^{C}$	$18,84^{C}$
100 – 200 days	Normal	$17,10^{A}$	$4,02^{A}$	$3,41^{A}$	$4,52^{A}$	$5,70^{A}$	$20,95^{A}$
	Subclinical	$16,94^{B}$	$4,10^{B}$	$3,45^{B}$	$4,44^{B}$	$8,05^{B}$	$20,41^{B}$
	Clinical	$16,88^{C}$	$4,13^{C}$	$3,48^{C}$	$4,35^{C}$	$9,93^{C}$	$20,34^{C}$
200 – 300 days	Normal	$16,38^{A}$	$4,27^{A}$	$3,64^{A}$	$4,49^{A}$	$5,98^{A}$	$21,62^{A}$
	Subclinical	$16,26^{B}$	$4,33^{B}$	$3,69^{B}$	$4,41^{B}$	$8,05^{B}$	$21,23^{B}$
	Clinical	$16,21^{C}$	$4,35^{C}$	$3,71^{C}$	$4,32^{C}$	$9,86^{C}$	$21,22^{C}$
> 300 days	Normal	$16,13^{A}$	$4,41^{A}$	$3,79^{A}$	$4,47^{A}$	$6,13^{A}$	$21,77^{A}$
	Subclinical	$16,00^{B}$	$4,47^{B}$	$3,84^{B}$	$4,38^{B}$	$8,05^{B}$	$21,53^{B}$
	Clinical	$15,94^{C}$	$4,49^{C}$	$3,86^{C}$	$4,28^{C}$	$9,83^{C}$	$21,54^{C}$

*LsMeans marked with different letter (capital or small), differ statistically highly ($p < 0.001$) significant or statistically ($p < 0.01$) significant
DMY – daily milk yield (kg), DFC – daily fat content (%), DPC – daily protein content (%), DLC – daily lactose content (%), SCClog – log value of somatic cell count, UREA – concentration of urea in milk (mg/dl)

The table presents the least squares means (LsMeans) of daily milk production parameters (DMY, DFC, DPC, DLC, SCClog, and UREA) in Simental cows, classified according to their health status (Normal, Subclinical, or Clinical Mastitis) and lactation stage classes (< 100 days, 100–200 days, 200–300 days, > 300 days). The results show how mastitis severity affects various milk production parameters and highlight the importance of managing animal health to optimize milk yield and quality.The general trend observed in the table is a decrease in daily milk yield as lactation progresses. Cows in the <100 days lactation stage have the highest DMY, while cows in the >300 days lactation stage produce the least milk. Health status significantly affects milk yield: normal cows consistently have the highest DMY across all lactation stages, followed by cows with subclinical mastitis, and finally, clinical mastitis cows, which exhibit the lowest milk yield. These differences are statistically significant ($p < 0.001$), suggesting that mastitis, particularly clinical mastitis, severely impacts milk production. Daily fat content follows a similar pattern to milk yield, with values decreasing slightly as lactation advances. For each lactation stage, clinical mastitis cows show the highest fat content, followed by subclinical mastitis cows, and normal cows, though the differences are small. These differences are statistically significant ($p < 0.01$). The higher fat content in mastitis-affected cows could be a compensatory mechanism, as the body attempts to adjust to the infection by altering the composition of milk.Daily protein content remains relatively stable across different lactation stages, with slight decreases observed in later lactation stages. For each lactation class, normal cows have the highest protein content, followed by subclinical mastitis cows, and clinical mastitis cows, which have the lowest protein content. These differences are statistically significant ($p < 0.01$), suggesting that mastitis affects the nutritional quality of milk, leading to lower protein levels, which can ultimately affect dairy product quality.

Lactose content in milk decreases slightly as lactation progresses. For each lactation stage, normal cows exhibit the highest lactose content, followed by subclinical mastitis cows, and then clinical mastitis cows. These differences are statistically significant ($p < 0.01$), indicating that mastitis impairs lactose production, a key component of milk.The log value of somatic cell count increases as lactation advances, with cows in the >300 days lactation stage showing the highest values. SCC is significantly higher in mastitis-affected cows. Clinical mastitis cows consistently have the highest SCClog values across all lactation stages, followed by subclinical mastitis cows, and normal cows, which have the lowest values. These differences are highly significant ($p < 0.001$), as somatic cell count is directly related to udder health and mastitis severity.Urea concentration in milk increases as lactation progresses, with the highest levels in the >300 days lactation stage. In terms of health status, cows with clinical mastitis show slightly higher urea concentrations than subclinical or normal cows, although the differences are less pronounced than for other parameters.

These differences are statistically significant ($p < 0.01$), and the elevated urea levels could reflect alterations in nitrogen metabolism due to the inflammatory response associated with mastitis.The results of this analysis demonstrate the significant negative impact of mastitis on milk production and quality. Cows with clinical mastitis have the lowest milk yield and milk quality, particularly in terms of protein and lactose content, and exhibit the highest somatic cell counts. Subclinical mastitis, although less severe, still results in decreased milk yield and quality. These findings underscore the importance of early detection and effective management of mastitis to prevent economic losses on dairy farms.The analysis also reveals that lactation stage plays a key role in milk production. As cows progress through lactation, there is a natural decline in milk yield and quality. This decline is further exacerbated by the presence of mastitis, particularly clinical mastitis, which highlights the need for appropriate management strategies to mitigate the effects of mastitis and maintain optimal milk production.

Table 2. LsMeans of daily milk production parameters regarding the Simentals health status and considering parity classes (I., II., III., and VI.+)

Parity class	Mastitis class	DMY	DFC	DPC	DLC	SCClog	UREA
I.	Normal	16,66^{A}	4,13^{A}	3,44^{A}	4,58^{A}	5,61^{A}	20,88^{A}
	Subclinical	16,56^{B}	4,21^{B}	3,50^{B}	4,50^{B}	8,04^{B}	20,28^{B}
	Clinical	16,52^{C}	4,25^{C}	3,52^{C}	4,42^{C}	9,89^{C}	20,10^{C}
II.	Normal	17,23^{A}	4,16^{A}	3,50^{A}	4,52^{A}	5,73^{A}	20,76^{A}
	Subclinical	17,01^{B}	4,24^{B}	3,55^{B}	4,44^{B}	8,07^{B}	20,27^{B}
	Clinical	16,94^{C}	4,26^{C}	3,57^{C}	4,36^{C}	9,91^{C}	20,09^{C}
III.	Normal	17,46^{A}	4,11^{A}	3,46^{A}	4,50^{A}	5,79^{A}	20,58^{A}
	Subclinical	17,22^{B}	4,18^{B}	3,52^{B}	4,42^{B}	8,04^{B}	20,13^{B}
	Clinical	17,13^{C}	4,22^{C}	3,54^{C}	4,34^{C}	9,90^{C}	20,10^{C}
VI.+	Normal	17,12^{A}	4,06^{A}	3,43^{A}	4,48^{A}	5,91^{A}	20,66^{A}
	Subclinical	16,93^{B}	4,13^{B}	3,48^{B}	4,39^{B}	8,08^{B}	20,32^{B}
	Clinical	16,83^{C}	4,16^{C}	3,51^{C}	4,29^{C}	9,98^{C}	20,32^{C}

*LsMeans marked with different letter (capital or small), differ statistically highly ($p < 0.001$) significant or statistically ($p < 0.01$) significant;
DMY – daily milk yield (kg), DFC – daily fat content (%), DPC – daily protein content (%), DLC – daily lactose content (%), SCClog – log value of somatic cell count, UREA – concentration of urea in milk (mg/dl)

Table 2 presents the least-squares means (LsMeans) for various daily milk production parameters in Simental cows, considering both health status (normal, subclinical, and clinical mastitis) and parity classes (I, II, III, and VI+). These parameters include daily milk yield (DMY), daily fat content (DFC), daily protein content (DPC), daily lactose content (DLC), log-transformed somatic cell count (SCClog), and urea concentration in milk (UREA). The results show that both health status and parity significantly influence milk production and composition, with clear patterns emerging from the data. Milk yield tends to decrease as parity increases, with the highest yields observed in first-lactation cows (I. parity) and the lowest in cows with higher parities (VI+). Within each parity group, normal cows produce the most milk, followed by subclinical, and then clinical mastitis cows. These differences are statistically significant ($p < 0.001$), highlighting that mastitis negatively affects milk production in all parity classes, with clinical mastitis having the most detrimental effect.For instance, in the I. parity group, normal cows yield 16.66 kg of milk per day, while subclinical and clinical mastitis cows yield slightly lower amounts (16.56 kg and 16.52 kg, respectively). As parity increases, milk yields remain higher for normal cows compared to those affected by mastitis, but the differences between health status groups narrow, particularly in the VI+ group, where the yield difference is minimal (16.83 kg for clinical mastitis cows vs. 17.12 kg for normal cows).Fat content (DFC) also shows a slight decrease as parity increases. Normal cows consistently show the highest fat content across all parity groups. However, the differences between health statuses are statistically significant ($p < 0.01$), with clinical mastitis cows consistently having the highest fat content, followed by subclinical and normal cows. For example, in the I. parity group, normal cows have a DFC of 4.13%, while clinical mastitis cows have 4.25%. This trend is consistent across all parity groups, indicating that mastitis may influence milk fat content, potentially as a compensatory response to the infection.Protein content (DPC) follows a similar pattern to fat content, with normal cows having the highest protein levels, followed by subclinical and clinical mastitis cows. These differences are statistically significant ($p < 0.01$). For example, in the I. parity group, normal cows produce milk with 3.44% protein, while clinical mastitis cows have 3.52% protein. As parity increases, protein content in milk generally decreases, but the relative differences between health status groups remain consistent. This suggests that mastitis, regardless of its form, slightly reduces milk protein levels, which could negatively affect milk quality.Lactose content (DLC) decreases with increasing parity, similar to protein and fat content. Normal cows again show the highest lactose content, while clinical mastitis cows exhibit the lowest levels. These differences are statistically significant ($p < 0.01$). In the I. parity group, normal cows produce milk with 4.58% lactose, while clinical mastitis cows have 4.42%. This pattern holds across all parity groups, suggesting that mastitis has a negative impact on lactose synthesis, which may reduce the nutritional value of the milk.Somatic cell count (SCC), a key indicator of udder health, is significantly higher in mastitis-affected cows across all parity groups. Clinical mastitis cows show the highest SCC levels, followed by subclinical cows, with normal cows having the lowest SCC values. These differences are highly statistically significant ($p < 0.001$). For instance, in the I. parity group, normal cows have a log-transformed SCC value of 5.61, subclinical cows have 8.04, and clinical mastitis cows have 9.89. As parity increases, SCC values also increase, reflecting the general decline in udder health as cows age, especially for those with mastitis. Elevated SCC levels in mastitis-affected cows indicate an ongoing immune response to infection, which impacts milk quality and yield.Urea concentration in milk tends to increase with increasing parity, and it is also higher in mastitis-affected cows compared to normal cows, although the differences are less pronounced than in other parameters. Statistically significant differences are observed ($p < 0.01$). For example, in the I. parity group, normal cows have a urea concentration of 20.88 mg/dl, while clinical mastitis cows have 20.10 mg/dl. While the differences in urea concentration are relatively small, they indicate changes in protein metabolism, which could be due to the metabolic stress associated with mastitis. This analysis reveals the significant impact of both health status and parity on daily milk production parameters in Simental cows. Mastitis, whether subclinical or clinical, consistently reduces milk yield, protein, and lactose content, while increasing fat content and somatic cell count. The detrimental effects of mastitis are more pronounced in cows with higher parities, although even first-lactation cows with clinical mastitis show a marked reduction in milk production and quality. Therefore, early detection and management of mastitis are critical for maintaining milk yield and quality, particularly in older cows. Regular monitoring of somatic cell count and milk composition is essential for managing udder health and ensuring optimal milk production in dairy herds.

Table 3. LsMeans of daily milk production parameters regarding the Simentals health status and considering animal production level (I., II., III., and VI.+)

Production class	Mastitis class	DMY	DFC	DPC	DLC	SCClog	UREA
I. DMY < 20 kg	Normal	9,43^{A}	4,27^{A}	3,60^{A}	4,45^{A}	6,06^{A}	20,31^{A}
	Subclinical	9,25^{B}	4,36^{B}	3,67^{B}	4,34^{B}	8,10^{B}	20,11^{B}
	Clinical	9,13^{C}	4,41^{C}	3,72^{C}	4,23^{C}	10,00^{C}	20,18^{C}
II. DMY in 20 – 30 kg	Normal	13,98^{A}	4,18^{A}	3,50^{A}	4,51^{A}	5,89^{A}	20,06^{A}
	Subclinical	13,94^{B}	4,25^{B}	3,56^{B}	4,43^{B}	8,08^{B}	19,70^{B}
	Clinical	13,91^{C}	4,27^{C}	3,58^{C}	4,33^{C}	9,96^{C}	19,67^{C}
III. DMY in 30 – 40 kg	Normal	17,96^{A}	4,09^{A}	3,44^{A}	4,54^{A}	5,74^{A}	20,76^{A}
	Subclinical	17,91^{B}	4,16^{B}	3,48^{B}	4,46^{B}	8,05^{B}	20,30^{B}
	Clinical	17,89^{C}	4,19^{C}	3,50^{C}	4,37^{C}	9,92^{C}	20,25^{C}
VI. DMY > 40 kg	Normal	26,14^{A}	3,98^{A}	3,34^{A}	4,57^{A}	5,47^{A}	21,91^{A}
	Subclinical	25,72^{B}	4,05^{B}	3,38^{B}	4,50^{B}	8,00^{B}	21,28^{B}
	Clinical	25,56^{C}	4,07^{C}	3,39^{C}	4,42^{C}	9,83^{C}	20,94^{C}

*LsMeans marked with different letter (capital or small), differ statistically highly ($p < 0.001$) significant or statistically ($p < 0.01$) significant;
DMY – daily milk yield (kg), DFC – daily fat content (%), DPC – daily protein content (%), DLC – daily lactose content (%), SCClog – log value of somatic cell count, UREA – concentration of urea in milk (mg/dl)

Table 3 provides the least-squares means (LsMeans) of daily milk production parameters in Simental cows, categorized by health status (normal, subclinical, and clinical mastitis) and animal production level. The production levels are classified based on daily milk yield (DMY) into four groups: less than 20 kg (I), 20–30 kg (II), 30–40 kg (III), and greater than 40 kg (VI+). The parameters analyzed include daily milk yield (DMY), daily fat content (DFC), daily protein content (DPC), daily lactose content (DLC), log-transformed somatic cell count (SCClog), and urea concentration in milk (UREA). The table demonstrates how mastitis and production levels influence milk composition and yield.As expected, cows in higher production classes (VI+) consistently produce more milk than those in lower production classes. For example, cows in class VI+ (DMY > 40 kg) yield 26.14 kg of milk per day when healthy, whereas cows in the I. class (DMY < 20 kg) yield only 9.43 kg per day. Additionally, mastitis (both subclinical and clinical) reduces milk yield within each production class, with clinical mastitis resulting in the most significant decrease in yield. For instance, in the I. production class, normal cows produce 9.43 kg of milk, while clinical mastitis cows produce 9.13 kg, a drop of approximately 0.30 kg. This trend is observed across all production levels, with clinical mastitis cows yielding less milk than those with subclinical or no mastitis. The differences in milk yield between health statuses are statistically significant ($p < 0.001$), emphasizing the negative impact of mastitis on milk production. Fat content in milk shows a similar trend to milk yield, with normal cows consistently exhibiting the highest fat content across all production levels. For example, in the I. production class, normal cows have 4.27% fat, while clinical mastitis cows have

4.41%. Although fat content increases slightly in mastitis-affected cows, the differences in fat content between health statuses are statistically significant ($p < 0.01$), with clinical mastitis cows showing the highest fat content. This may be due to metabolic changes associated with mastitis, potentially as a compensatory response. In higher production classes, this trend continues, with clinical mastitis cows generally showing the highest fat content compared to subclinical and normal cows. Protein content in milk follows a similar pattern, with normal cows consistently having the highest protein levels in all production classes. For instance, in the I. production class, normal cows have 3.60% protein, while clinical mastitis cows have 3.72%. The differences in protein content are statistically significant ($p < 0.01$), and clinical mastitis cows generally have higher protein content compared to subclinical cows, despite the negative effect of mastitis on overall milk yield. As with fat content, protein content tends to be slightly higher in clinical mastitis cows across all production classes, indicating that mastitis might lead to changes in milk composition. Lactose content shows a general decline with increasing severity of mastitis. Normal cows exhibit the highest lactose content, while clinical mastitis cows show the lowest levels. For example, in the I. production class, normal cows have 4.45% lactose, while clinical mastitis cows have 4.23%. These differences are statistically significant ($p < 0.01$). As mastitis severity increases, lactose synthesis appears to decrease, which could contribute to the reduced nutritional value of milk. The same trend is observed across higher production levels, with clinical mastitis cows having the lowest lactose content. Somatic cell count (SCC), which is a key indicator of udder health, shows a clear increase with the severity of mastitis. Clinical mastitis cows have the highest SCC, followed by subclinical cows, and normal cows show the lowest SCC. The differences in SCC are highly significant ($p < 0.001$). For instance, in the I. production class, normal cows have a log-transformed SCC of 6.06, while clinical mastitis cows have 10.00. As production level increases, SCC also increases, especially for mastitis-affected cows. This indicates that udder health declines as milk production rises, which is expected as higher-yielding cows often experience more udder stress. Urea concentration in milk generally increases with production level, with higher-yielding cows having higher urea concentrations. For example, in the VI+ production class, normal cows have 21.91 mg/dl of urea, while clinical mastitis cows have 20.94 mg/dl. The differences in urea concentration are statistically significant ($p < 0.01$), though the differences between health statuses are relatively small compared to other parameters. This suggests that mastitis has a moderate effect on milk protein metabolism, reflected in the urea concentration in milk. The analysis of Table 3 highlights the substantial impact of both mastitis and production level on milk production parameters in Simental cows. Mastitis, whether subclinical or clinical, consistently reduces milk yield, protein content, and lactose content, while increasing fat content and somatic cell count. Higher production levels are associated with higher milk yields but also with higher somatic cell counts and slightly higher urea concentrations in milk. Clinical mastitis has the most significant negative effect on milk yield and quality, and this impact is evident across all production classes. Effective management and early detection of mastitis are crucial for maintaining optimal milk production and quality, especially in high-producing cows. Regular monitoring of somatic cell count and milk composition is essential for ensuring udder health and maximizing dairy farm profitability.

The results of this study show a significant impact of mastitis on daily milk production and milk quality in Simmental cows. In all lactation stages and production classes, cows with clinical mastitis had lower daily milk yields compared to healthy cows, while cows with subclinical mastitis also showed a decrease in production, but to a lesser extent. These results confirm previous studies indicating significant milk production losses due to mastitis, especially subclinical mastitis, which is responsible for long-term losses (Halasa *et al*., 2007). Furthermore, the data suggest that older cows and cows in later stages of lactation have lower daily milk yields, which is linked to cumulative udder stress. Higher SCC in these groups confirms the negative effect of stress on the udder (Schukken *et al*., 2003). Changes in milk composition, such as increased fat and protein content, along with decreased lactose levels, were also observed in cows with mastitis, which can negatively affect milk quality (Nir, 2003; Seegers *et al.,* 2003). In conclusion, mastitis, whether clinical or subclinical, has a significant impact on milk production and quality. Preventive management of mastitis, including SCC monitoring, better hygiene practices, and nutritional support, is essential for reducing production losses and maintaining milk quality (Argaw, 2016; Kamal *et al*., 2014; Özkan Gülzari *et al*., 2018).

CONCLUSIONS

Based on the results of this study, it can be concluded that mastitis, both clinical and subclinical, has a significant impact on daily milk production and milk quality in Simmental cows. Cows with subclinical mastitis also showed a reduction in milk production compared to healthy cows, although this reduction was less pronounced than in those with clinical mastitis. Data from different stages of lactation and production levels clearly indicate a negative effect of mastitis on daily milk yield, especially in older cows and those in the later stages of lactation, which is associated with cumulative udder stress.

Additionally, changes in milk composition, including increased fat and protein content and reduced lactose levels, further confirm the negative impact of mastitis on milk quality, which can have long-term consequences on farm economics. Therefore, early detection and effective management of mastitis, including monitoring SCC, improving hygiene practices, and proper nutrition, are crucial for reducing milk production losses and preserving milk quality.

With proper prevention and control of mastitis, it is possible to reduce economic losses and optimize milk production, thereby enhancing the sustainability and profitability of dairy farms.

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ZDRAVSTVENO STANJE I NJEGOVI UČINCI NA DNEVNU PROIZVODNJU MLIJEKA U SIMENTALSKIH KRAVA UZIMAJUĆI U OBZIR KLJUČNE FAKTORE VEZANE UZ ŽIVOTINJE

Sažetak

Ova studija analizirala je utjecaj mastitisa na dnevne parametre proizvodnje mlijeka u Simmental krava, uzimajući u obzir faktore poput faze laktacije, pariteta i razine proizvodnje. Nakon primjene mjera kontrole kvalitete, konačni skup podataka sastojao se od 2,835,101 zapisa o testnim danima Simmental krava. Rezultati su pokazali da su i subklinički i klinički mastitis negativno utjecali na prinos mlijeka i kvalitetu mlijeka. Krave sa subkliničkim mastitisom imale su manji prinos mlijeka u usporedbi s zdravim kravama, dok su one s kliničkim mastitisom pretrpjele još veće gubitke. Mastitis je također utjecao na kvalitetu mlijeka, s povećanjem sadržaja masti i proteina, te smanjenjem razine laktoze. Učinci su bili izraženiji kod krava u kasnijim fazama laktacije i onih s višim razinama proizvodnje. Rana detekcija i intervencija ključni su za minimiziranje gubitaka u proizvodnji mlijeka i očuvanje kvalitete mlijeka. Poboljšanje praksi u upravljanju farmom, kao što je praćenje somatskih stanica i osiguranje odgovarajuće higijene i prehrane, ključno je za optimizaciju proizvodnje mlijeka i smanjenje ekonomskih gubitaka.

Ključne riječi: *mastitis, mliječna goveda, Simentalac, proizvodnja mlijeka, somatske stanice*

DETERMINATION OF NUTRIENT DIGESTIBILITY OF TOTAL MIXED RATIONS IN THE RUMEN USING *IN VITRO* METHOD*

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Original scientific paper

Abstract

The digestibility rate is a crucial indicator of nutrient utilization in dairy cow diets. We can accurately determine ruminant feed digestibility in an artificial rumen using in vitro fermentation. This involves incubating a feed sample with liquid rumen under anaerobic conditions using a buffer solution that simulates rumen saliva. After approximately 48 hours of fermentation, the remaining dry matter was measured, followed by the quantification of digested cellulose, produced gases, and volatile fatty acids. Commercial complete rations (TMR) were used in the experiment to ensure uniform consumption in both quantitative and qualitative terms. The research aimed to determine the digestibility rate and kinetics of dry matter and nutrient components in TMR samples designed for milk production in different lactation stages. The results showed significant differences in the composition of the mixtures and the same indicators after incubation through eight different time intervals. The research confirmed that digestibility analysis with the ANKOM incubator provided accurate, reproducible, and cost-effective results. Collaborative studies also yielded desirable results despite differences in fermentation factors.

Keywords: *In vitro digestibility, total mixed rations (TMR), dairy cows*

INTRODUCTION

Ruminants, such as cattle, can efficiently digest rations rich in coarse forage due to their unique digestive system. Ruminal fermentation is a continuous process due to the constant turnover of microorganisms in the rumen (Valentine et al., 1994). The *in vitro* dry matter digestibility (IVDMD) method is commonly used to evaluate the nutritional value of ruminant feeds. As a closed system, the *in vitro* digestibility method uses simulated digestive fluids and enzymes to study feed digestion. An artificial incubator with a thermostatic chamber at 39°C and four rotating jars was used to measure the in vitro digestibility of feeds. The Ankom Daisy® (Ankom Technology, Macedon, NY, USA) apparatus allows simultaneous incubation of different feedstuffs in sealed bags, making the process more efficient. The method is based on Tilly and Terry's (1963) and

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IVDMD. Since then, the technique has been modified (Goering and Van Soest, 1970; Ammar *et al*., 1999). Modifications utilized by various researchers include different rumen (fluid) inoculum, buffer solutions, sample size and weight, and beg type, which have been developed to adjust the pH of the inoculum and equilibrate fermentation processes (Mabjeesh *et al*., 2000). Due to costs, labor-intensive and ethical considerations, alternative *in vitro* analysis is preferred and widely used (Tassone *et al*., 2020). It provides accurate data for predicting ruminant digestibility. This scientific work focuses on researching the digestibility rates and kinetics of dry matter and individual nutrient components in samples of commercial TMR rations in the rumen using the *in vitro* method.

MATERIALS AND METHODS

The study utilized original commercial total mixed ration (TMR) samples and rumen fluid, collected from lactating cows at various stages, obtained from the dairy farm "KJP PD Bojnik" in Sarajevo (https://pdbutmir.com). These rations were formulated to meet their maintenance and production requirements (NRC, 2001). The TMR rations were composed of corn silage, grass-legume mixture hay, alfalfa haylage, concentrate mixture (22% crude protein), super concentrate mixture (38% CP), brewers grains, limestone, salt, sodium bicarbonate and premix (Table 1). All preparations and procedures were conducted at the Animal Feeds and Feeding laboratory of the Faculty of Agriculture and Food Sciences (University of Sarajevo). The pre-dried samples (at 55ºC for 16 hours) were finely ground in a Foss Hammertec™ hammer (Foss Analytics, Hillerod, Denmark) mill through a ~2.0 mm screen. Approximately 0.5±0.05 g of the sample was weighed, placed, and encapsulated (heat sealed) in the filter (acetone pre-rinsed) bags (F57, polyester, with a porosity of 25 μm). Three Simmental dairy cows were used as rumen fluid donors (with one cow representing each of the three production phases). Rumen fluid was collected using a vacuum pump connected to a glass container and fitted with a semiflexible ~1.8-meter-long oro-ruminal probe. The probe was inserted into the oro-ruminal tract within the ventral sac of the rumen. Approximately 2,0 liters of rumen inoculum was collected using the UPS-NS vacuum esophageal probe (Lalić *et al*., 2001) using the probing technique 4 hours after the morning consumption. The initial 500 ml of inoculum was discarded because of potential saliva contamination (Raun and Burroughs, 1962). The pH of the rumen liquor was measured right after collection, and it was found to be 6.67. The liquid content of the rumen was transferred to a sterile, prewarmed (40.0±1.0 °C) container and immediately transported to the laboratory. Up to 25 samples were transferred into each digestion jar. Then, 400 ml of inoculum (pre-filtered through four layers of cheesecloth) was added to the buffer solution (artificial saliva). Buffer solutions A (KH_2PO_4 (10.0 g/L), $MgSO_4 \times 7H_2O$ (0.5 g/L), NaCl (0.5 g/L), $CaCl_2 \times 2H_2O$ (0.1 g/L), Urea (0.5 g/L)) and B (Na_2CO_3 (15.0 g/L), $Na_2S \times 9H_2O$ (1.0 g/L)) are prepared by combining 266 ml of solution B with 1330 ml of solution A in a 1:5 ratio to create a total volume of 1600 ml. The mixture was purged with CO_2 gas for thirty seconds and the lid (with a check valve) was sealed. The prepared jars were incubated at 39±0,5 ºC for 3, 6, 12, 24, 48, 72, and 96 hours (Daisy II Incubator, Ankom Technology, Macedon, NY, USA). Each sample was incubated in three replicates. After incubation, the bags are removed and rinsed in tap (cold) water.

Also, applying a Neutral Detergent Solution is essential to remove any microbial debris or remaining soluble fractions. Then, they are soaked in acetone, rinsed again and dried in an oven at 105°C until a constant mass is achieved (up to two hours). The bags are then weighed and the *in vitro* dry matter digestibility (IVDMD) is calculated based on the amount of dry matter that disappeared from the initial weight inserted into the bag.

Samples of complete (TMR) mixtures were analyzed before and after incubation to determine the content of individual nutrients. The analyses included determining dry matter (DM) and moisture content (TM) by drying (Memmert UE 500; ISO 6496:1999), crude ash (Ash) and organic matter (OM) by incineration (Nabertherm 7/H; 5984:2002), crude protein (CP) by combustion (Foss TecatorTM; ISO 5983-1:2005), distillation and titration (KjeltecTM 2200; 5983-2:2005) and neutral (sodium sulfite; α-amylase) and acid detergent fiber (ADF) content by extraction (Ancom A200; ISO 16472:2006 and 13906:2008) based on Van Soest et al. (1991) and Mertens (2002).

Starch (ST) content was determined polarimetrically (ISO 6493:2000). Calculation of *in vitro* DM, CP, NDF and ADF digestibility content was performed using the following formula (1), where W_1 is bag mass (g), W_2 is sample mass (g), W_3 is bag and sample residual mass (g), C_1 is blank bag correction (final/initial bag mass).

$$(1) \quad IVDMD = \frac{100 - \left(W_3 - (W_1 \times C_1)\right) \times 100}{W_2}$$

Table 1. Ingredients, chemical composition and daily intake of commercial TMR rations fed to the cows on a farm

Ingredient composition (% DM[5])	**1st phase of lactation**	**2nd phase of lactation**	**3rd phase of lactation**
Corn silage	40.6	45.5	54.9
Grass-legume mix. hay	11.7	17.2	19.2
Alfalfa haylage	2.2	6.9	10.8
Concentrate mixture (22% CP)[1]	34.8	29.1	13.6
Concentrate mixture (38% CP)[2]	3.9	-	-
Brewers grains	5.5	-	-
Limestone	0.1	0.3	0.4
Salt	0.3	0.2	0.2
Sodium bicarbonate	0.2	-	-
Premix[3]	0.7	0.8	0.9
F:C[4]	54:46	69:31	85:15
Chemical composition (% of DM)			
Dry matter	47.2	41.8	40.5
Crude ash	6.61	6.33	5.83
Organic matter	93.39	93.67	94.17
Crude protein	12.93	10.17	9.08
Neutral detergent fiber	52.26	50.88	53.39
Acid detergent fiber	21.97	22.10	21.58
Starch	30.06	34.77	30.14
Daily intake (g/day)			
Dry matter	22.700	18.200	16.300
Crude protein	2.950	1.850	1.480
Neutral detergent fiber	11.863	9.260	8.705
Acid detergent fiber	4.987	4.022	3.517
Starch	6.825	6.328	4.913

[1]cereal, by-products of the oil and fermentation industry, by-products of the mill industry, dried plant products, macroelements, microelements, vitamins; crude protein min. 22%, crude fiber max.12%, ash max. 10%, Ca 0,72%, P 0,92%, Na 0,20%, Vit.A 10.000 IU/kg, Vit.D_3 2.000 IU/kg, Vit. E 20 mg/kg;

[2]by-products of the mill industry, oilseed meal, alfalfa meal, salt, limestone, dicalcium phosphate, mineral and vitamin supplement, crude protein nim. 37%, crude fiber max. 10%, ash max. 12%, P min. 1,8%, Na min. 0,6%, vit.A 30.000 IU, vit.D_3 4.000 IU, vit.E 50 mg, Fe 80 mg, Cu 15 mg, Mn 50 mg, Zn 50 mg, Mg 80 mg, I 1,8 mg, Se 0,3 mg, Co 0,2 mg;

[3]Vitamins, methionine, salt, monocalcium phosphate, magnesium oxide, limestone, sugar, magnesium sulfate, Na 5,0%, P 1,0%, Ca min. 15%, Mg 3%, S 0,5%, methionin 1%, sugar 5%, vit.A 220.000 IU, vit.D_3 40.000 IU, vit.E 1.500 mg, vit.B_1 21 mg, vit.B_2 10 mg, vit.B_6 4 mg, vit.B_{12} 0.5 mg;

[4] forage to concentrate ratio;

[5] DM – dry matter

The pH of the ruminal fluid was measured immediately after each incubation endpoint (pH700 Eutech Instruments, Singapore). Table 1 shows TMR´s ingredients, structure, chemical composition, and estimated consumption of complete rations (based on dry matter). The obtained data were analyzed using descriptive and comparative methods using a statistical software package (IBM SPSS Statistics *ver.* 23.0). The feed and animal nutrition laboratory conducted complete sample preparation and analyses.

RESULTS AND DISCUSSION

The entire process of setting up the experimental trial was carried out according to the guidelines provided by the manufacturer of the Daisy incubator. This involved optimizing various environmental factors such as temperature, anaerobic conditions, mixing, and the amount and ratio of the artificial saliva-buffer solution to ensure proper and continuous fermentation. This is supported by the fact that the pH value did not deviate significantly from the usual values. The initial value (6.60) did not change compared to the endpoint of incubation (96 hours), ranging from 6.38 to 6.60 throughout the studies shown in Table 2. The digestibility of dry matter is a critical factor in determining the nutritional value of a feed. The results for the IVDMD revealed significant variations in complete meals during incubation (Table 2). The values ranged from 25.84 to 27.20% at the third hour and increased to 77.48% and 79.58% by the 96th hour of measurement. Tassone *et al*. (2023) published similar results in their 48-hour dry matter digestibility research. No significant differences were observed among the meals during specific measurement periods. Kahraman *et al*. (2023) reported a slight decrease in total mixed rations (TMR) digestibility when different feed ingredients were used to prepare mixed rations. Furthermore, Paul *et al.* (2023) observed a more significant decrease in the digestibility of TMR during the 24-hour and 48-hour incubation periods compared to the findings of this study.

Table 2. Average values of in vitro digestibility of DM, CP, NDF and ADF throughout the incubation period (mean±sd)

Item	**Period of incubation**							
Hour	3^{00}	6^{00}	12^{00}	24^{00}	48^{00}	72^{00}	96^{00}	**P***
pH	6.48	6.50	6.49	6.48	6.47	6.38	6.60	
Digestibility coefficient of dry matter (%)								
TMR$_1$ (n=6)	27.20^{aA} ±0.87	34.59^{bA} ±1.31	53.45^{cA} ±1.46	57.35^{dA} ±0.77	66.84^{eA} ±1.34	70.74^{fA} ±0.21	77.48^{gA} ±1.02	0.000
TMR$_2$ (n=6)	27.10^{aA} ±0.77	30.60^{bA} ±4.34	50.43^{cA} ±3.03	57.49^{dA} ±0.44	68.04^{eA} ±2.16	71.27^{fA} ±0.52	78.73^{gA} ±1.22	0.000
TMR$_3$ (n=6)	25.84^{aA} ±1.40	33.16^{bA} ±0.62	51.17^{cA} ±1.22	54.79^{dA} ±2.95	66.59^{eA} ±0.51	70.80^{fA} ±0.76	79.58^{gA} ±1.34	0.000
P**	0.282	0.252	0.254	0.192	0.489	0.477	0.181	

			Digestibility coefficient of crude protein (%)					
TMR$_1$ (n=6)	33.63^{aA} ±1.44	34.28^{aA} ±0.95	38.19^{bA} ±0.57	49.14^{bA} ±1.89	53.98^{cA} ±1.59	66.57^{dA} ±1.32	85.74^{eA} ±1.46	0.000
TMR$_2$ (n=6)	32.09^{aA} ±1.57	32.33^{aA} ±0.74	35.90^{bB} ±0.88	38.66^{cA} ±1.30	51.80^{dA} ±1.72	66.96^{fA} ±1.04	84.39^{gA} ±1.35	0.000
TMR$_3$ (n=6)	15.76^{aB} ±0.70	16.02^{aB} ±1.43	18.17^{aC} ±0.59	29.33^{bB} ±0.93	46.22^{cB} ±0.97	62.94^{dA} ±2.91	84.61^{fA} ±1.36	0.000
P**	0.000	0.089	0.000	0.000	0.002	0.082	0.492	
			Digestibility coefficient of neutral detergent fiber (%)					
TMR$_1$ (n=6)	8.35^{aA} ±1.44	14.40^{bA} ±1.84	32.86^{cA} ±2.40	43.07^{dA} ±0.48	55.68^{eA} ±0.80	57.71^{eA} ±0.27	63.31^{fA} ±2.55	0.000
TMR$_2$ (n=6)	3.67^{aB} ±1.23	11.48^{bAB} ±1.63	27.39^{cB} ±1.32	36.05^{dB} ±0.71	55.04^{eA} ±3.88	57.70^{eA} ±2.48	65.87^{fA} ±1.96	0.000
TMR$_3$ (n=6)	3.89^{aB} ±1.74	10.91^{bB} ±1.55	29.83^{cAB} ±0.93	35.06^{dB} ±0.91	53.39^{eA} ±1.78	60.42^{fA} ±0.98	66.50^{gA} ±2.49	0.000
P**	0.011	0.089	0.020	0.000	0.548	0.121	0.538	
			Digestibility coefficient of acid detergent fiber (%)					
TMR$_1$ (n=6)	-	-	15.56^{aA} ±1.60	20.98^{bA} ±0.44	33.86^{cA} ±1.76	38.22^{dA} ±1.65	45.89^{eA} ±0.73	0.000
TMR$_2$ (n=6)	-	-	12.82^{aB} ±1.09	17.03^{bB} ±0.46	34.86^{cA} ±3.00	39.70^{dA} ±4.64	51.38^{fB} ±1.54	0.000
TMR$_3$ (n=6)	-	-	6.85^{aC} ±0.29	16.59^{bB} ±1.47	34.63^{cA} ±3.44	40.93^{dA} ±1.01	54.22^{fC} ±1.57	0.000
P**	-	-	0.000	0.002	0.904	0.553	0.001	

DM – dry matter; CP – crude protein; NDF – neutral detergent fiber; ADF – acid detergent fiber; TMR$_{1,2,3}$ - total mixed rations according to the phase of lactation (early, mid and late)

* The mean difference between incubation periods (Significance was declared at $P < 0.05$)

** The mean difference between TMR rations inside incubation periods (Significance was declared at $P < 0.05$)

Figure 1 illustrates the dynamics of dry matter digestibility and associated regression models, which exhibit a very high coefficient of determination (R^2). No differences between dry matter digestibility (DMD) and TMR rations were observed for any time point (Table 2).

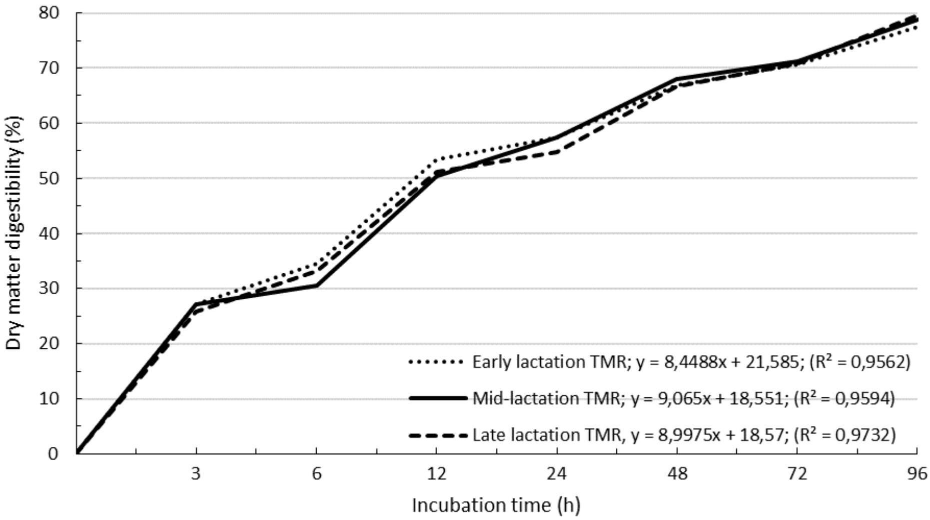

Figure 1. Dry matter digestibility rates of different TMR rations

The crude protein content in feed mixtures is primarily determined by the nitrogen levels needed to meet the requirements of dairy cows for synthesizing body and milk protein. By incubating samples, we assessed the average raw protein digestibility in the tested TMR throughout the incubation period. The results showed significant variations in crude protein digestibility among the complete meals during the incubation (Table 2). The digestibility values ranged from 32.69%, 30.34%, and 15.84% at the third hour to 85.89%, 83.58%, and 82.98% at the 96th hour of incubation for TMR_1, TMR_2, and TMR_3, respectively. The first and second TMR mixtures consistently exhibited higher raw protein digestibility throughout the incubation period. This can be attributed to the differences in soluble protein content in the mixtures before incubation and the substantial contribution of protein from concentrates, which is naturally more digestible. In contrast, the crude protein digestibility of the third TMR mixture is hindered by a significant amount of crude protein being bound to structural carbohydrates due to the more substantial proportion of bulky feed components, making it less accessible. Although this study did not cover this, it would be valuable to investigate the individual crude protein fractions and their digestibility. Furthermore, the digestibility of raw protein in the rumen is influenced by the level of productivity and intensity, which are closely tied to the retention time of feed in the rumen and the quality and physical characteristics of feed particles (including size and distribution of effective fibers). An evident difference existed in all rations between the defined incubation periods ($P<0.05$). Significant differences in the digestibility of crude protein between meals were determined, except in the 72nd and 96th hour of incubation ($P>0.05$). In most incubation points, differences did not exist between the first two rations, while differences appeared mainly between the first, second and third rations. The following figure 2 illustrates the dynamics of raw protein digestibility and includes associated regression models with a high coefficient of determination (R^2).

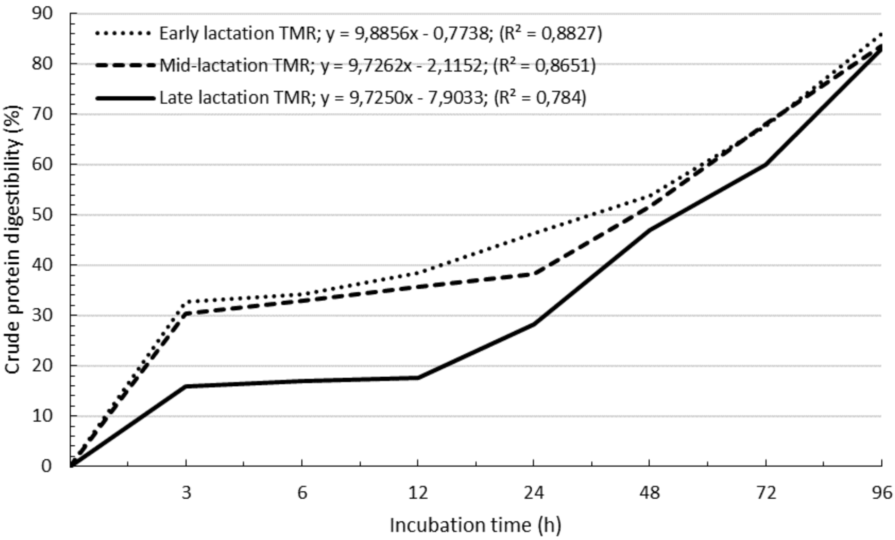

Figure 2. Crude protein digestibility rates of different TMR rations

The stagnation in the digestibility of raw protein from the 3rd to the 12th hour of incubation is closely associated with the digestibility of NDF and ADF, which refers to the protein bound to fibers (B fraction). A significant positive trend in digestibility continues to increase until the 96th hour, reaching approximately 80% across all three meals (Figures 2, 3, and 4).

Neutral detergent fibers (NDF) are the most common measure and represent the more easily digestible fraction of structural fibers, specifically hemicellulose (Mertens, 1977). The nutritional value of a ruminant feed or mixture is assessed based on its NDF content and potential digestibility. These factors significantly depend on the developmental stage of the plant material and the preservation method used. Previous studies on total mixed ration (TMR) consumption in cows indicate that mixtures with higher NDF digestibility lead to greater dry matter intake, positively correlated with higher milk yields (Oba and Allen, 1999; Spanghero and Zanfi, 2009). Notably, the results from the NDF digestibility tests showed significant variations among complete meals throughout the incubation period (Table 2). The values ranged from 8.52%, 3.75%, and 4.29% during the third hour to 63.88%, 65.88%, and 67.63% in the 96th hour of incubation. High-producing cows usually retain feed particles in their rumen for no more than 48 hours. However, recent measurements suggest that this duration may have decreased to 24 and 30 hours. This variation depends on the intensity of the cow's production and the quality (digestibility) of the forages they consume (Oba and Allen 1999). It is explained that shorter incubation times are more effective in assessing the digestion potential of NDF in high-producing lactating dairy cows. Compared to reference values for TMR rations, these results categorize the rations as medium digestible (54.4% - 57.1%), according to Hoffman and Combs (2008). As shown in Table 2, the NDF digestibility is significantly lower in TMRs consumed by cows in the third stage of lactation due to a higher proportion of the fibrous component in the diet. Therefore, these rations are classified as poorly digestible based on reference values (45.7% - 53.9%). However, by the 72nd and 96th hours of incubation, the digestibility significantly improves for the third TMR ration. This increase may be attributed to the longer retention time of the meals in the rumen, which enhances the digestibility of both dry matter and NDF due to prolonged exposure to decomposition processes (microbial activity). The following figure illustrates the dynamics of NDF digestibility, featuring associated regression models with a very high coefficient of determination (R^2).

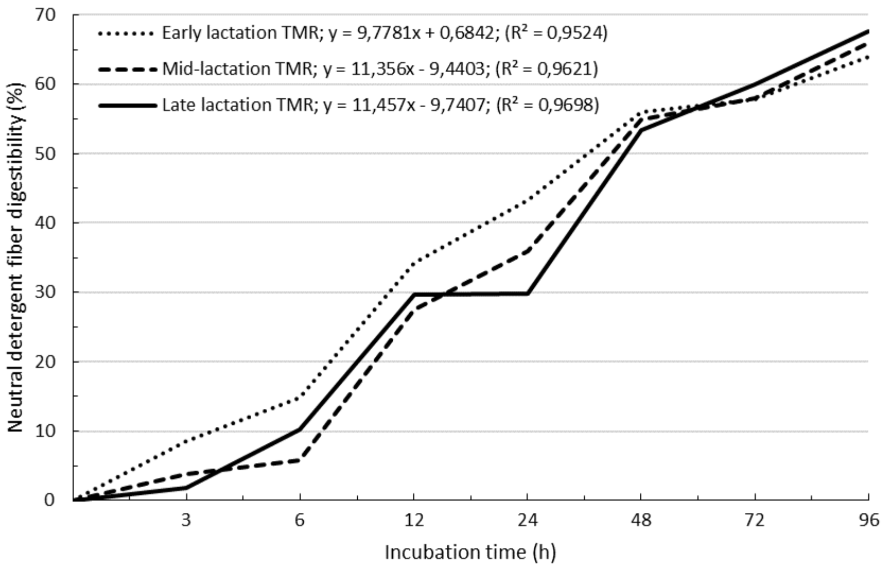

Figure 3. NDF digestibility rates of different TMR rations

The recommendations for ADF are primarily based on the needs of NDF due to their close relationship. Given the strong correlation, ADF and NDF, along with energy values, can be calculated using regression equations. Measuring ADF is essential when assessing forages of meager nutritional value, such as poor-quality hay or straw. Acid detergent fibers (ADF) represent the heavier and insoluble fiber fraction, primarily cellulose and lignin. Consequently, ADF is considered a reliable indicator of the potential digestibility of feeds or their mixtures. As anticipated, the results of ADF digestibility revealed significant variations among the complete meals throughout the incubation period (Table 2). The digestibility values ranged from 15.37%, 12.81%, and 6.70% at the 12th hour to 45.56%, 51.48%, and 54.22% at the 96th hour. It is important to note that digestibility values for the third and sixth hours of incubation are missing. ADF tends to show measurable values only in the later stages of incubation, beginning at the 12th hour. Furthermore, there is a clear positive trend in digestibility, demonstrating significant differences between the rations across various incubation periods (figure 4).

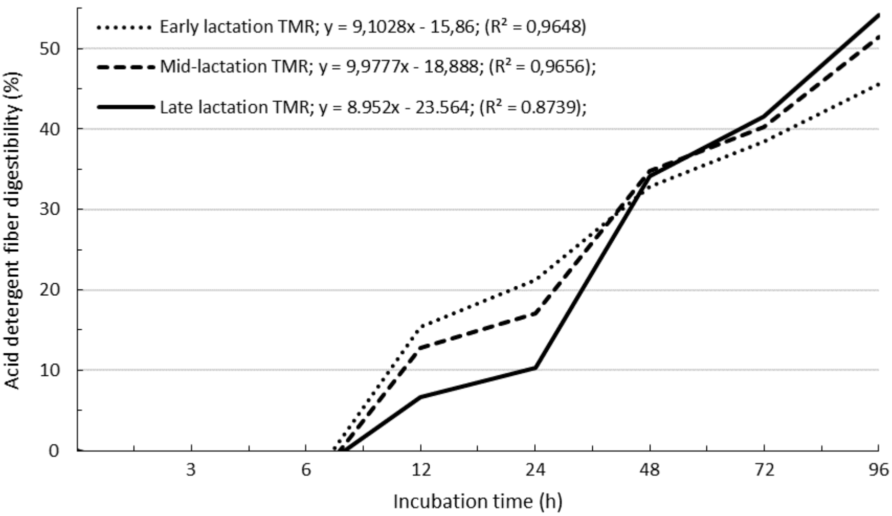

Figure 4. ADF digestibility rates of different TMR rations

CONCLUSIONS

The *in vitro* digestibility method was developed as a cost-effective and ethical alternative to the more expensive and time-consuming *in vivo* method for evaluating nutrient digestibility in ruminants and non-ruminants. This study confirms significant correlations established in some earlier research. Regarding the quality of total mixed rations, particularly for cows in peak lactation, it was noted that their crude protein content is slightly lower than the recommended for optimal production (NRC, 2001). As anticipated, the results demonstrated notable differences in the chemical composition of the complete mixtures. Additionally, variations were observed in critical indicators following incubation over eight different time intervals, affecting dry matter, crude protein and neutral and acidic detergent fibers. Based on the research conducted on digestibility and nutritional value assessment, it is evident that there is potential for enhancing the quality of feed, particularly for those used during the first and second stages of lactation.

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ODREĐIVANJE PROBAVLJIVOSTI HRANJIVIH MATERIJA KOMPLETNIH OBROKA U BURAGU *IN VITRO* METODOM

Sažetak

Stepen probavljivost predstavlja najbitniji pokazatelj iskorištenosti hranjivih materija u ishrani mliječnih krava. U vještačkom buragu, upotrebom *in vitro* fermentacije, moguće je relativno precizno odrediti probavljivost krmiva za preživare. Ovaj postupak uključuje inkubaciju uzorka krmiva s tekućim sadržajem buraga u anaerobnim uslovima. Kao medij koristi se puferni rastvor koji simulira sastav pljuvačke u buragu. Fermentacija traje otprilike 48 sati, nakon čega se mjeri preostala suha materija, probavljena celuloza, proizvedeni plinovi i hlapljive masne kiseline. Kao materijal u radu, korišteni su komercijalni kompletni obroci (TMR) za ishranu krava, što omogućuje da konzumacija bude ujednačenja u kvantitativnom i kvalitativnom smislu. Ideja istraživanja je bila da se utvrdi stepen i kinetika probavljivosti suhe materije i pojedinačnih hranjivih komponenata u uzorcima komercijalnih TMR (kompletno miješanih) obroka, dizajniranih prema realnim proizvodnim potrebama proizvodnje mlijeka u prvom, drugom i trećom stadiju laktacije. Rezultati su pokazali značajne razlike u fizičko-hemijskim sastavu kompletnih smjesa, ali i u istim pokazateljima nakon inkubacije kroz osam različitih vremenskih intervala kako za suhu materiju, tako i za sirovi protein, neutralna i kisela deterdžentska vlakna. Ovo istraživanje je samo potvrdilo da analiza probavljivosti sa ANKOM inkubatorom je pokazala zavidne rezultate po pitanju tačnosti, ponovljivosti i troškova sprovođenja generalno. Mnoge kolaborativne i simultane studije pokazale su zavidne rezultate, uprkos činjenici što postoje značajne razlike u pogledu osiguranja biotskih i abiotskih faktora fermentacije.

Ključne riječi: *probavljivost in vitro, ukupni miješani obroci (TMR), mliječne krave*

UTICAJ FIZIČKE EFEKTIVNOSTI VLAKANA NA KONZUMACIJU I SELEKTIVNOST KOMPONENATA TMR OBROKA ZA KRAVE U LAKTACIJI*

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Originalni naučni rad - *Original scientific paper*

Sažetak

Kompletno miješani obrok (TMR) se sastoji od velikog broja različitih nutrijenata sadržanih u krmivima različitog porijekla, određene količine i sastava, izmiješanih u jedan obrok. Ovakav način pripreme obroka počeo se koristiti zbog sposobnosti životinja da biraju dijelove obroka u toku ishrane, preferirajući sitnije i probavljivije čestice. Korištenjem TMR obroka životinjama se ograničava mogućnost selektovanja hrane, posebno voluminoznih krmiva koja su veoma značajna u procesu probave i razgradnje hrane. Fizički efektivna vlakna su važna zbog sprječavanja različitih zdravstvenih poremećaja, aktivnosti žvakanja, buražnih fermentacija, pH vrijednosti buražnog sadržaja, ali i sadržaja masti u mlijeku. Iako je TMR kompletan obrok, različitim analizama utvrđeno je da ipak dolazi do pojave selektivnosti od strane krava u srednjoj fazi laktacije koje su u najvećoj mjeri konzumirale sitne probavljivije čestice hrane. Sadržaj $peNDF_{8mm}$ i $peNDF_{4mm}$ se kroz period ishrane kretao od 12,92 do 25,99% odnosno 23,25% do 37,23%. Ovakva dinamika ishrane pokazala je da se u prvim satima ishrane najviše konzumira koncentratni dio TMR obroka, a zatim voluminozni dio koji potiče žvakanje i preživanje hrane. Ovakav način konzumiranja hrane je posljedica neravnomjerne ishrane nakon čega dolazi i do promjena uslova probave u buragu. Analizom eksperimentalnog TMR obroka dokazan je voluminozni karakter obroka koji je na osnovu prosječne dužine čestica svrstan u fino mljeveni obrok. Pored navedenih analiza, za preciznije procjene vrijednosti fizičke efektivnosti TMR obroka mogu se uključiti i dodatna ispitivanja, kao što su mjerenje pH vrijednosti u buragu, te mjerenje sadržaja različitih parametara mlijeka, posebno mliječne masti.

Keywords: *kompletno miješani obrok (TMR), selektivnost, fizički efektivna vlakna, fizička efektivnost*

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UVOD

Normirana ishrana životinja kao dio menadžmenta u intenzivnom uzgoju životinja je preduslov visoke produktivnosti i dobrog zdravlja. U cilju osiguranja izbalansiranog obroka, još od 1950. godine, počeo se koristiti kompletno miješani obrok ili TMR (Total mixed ration). Kompletno miješani obroci u tom smislu predstavljaju racionalno rješenje koji na najefikasniji i najprofitabilniji način osigurava sve ishrambene potrebe za održivu proizvodnju mlijeka. Kompletno miješani obrok je sveobuhvatan koncept savremenog načina držanja goveda koji zahtijeva specijalizovane i specifične uslove i kapacitete u intenzivnim uslovima proizvodnje na farmi. Primarna namjena TMR obroka je formiranje funkcionalne homogene smjese sastavljene od svih neophodnih nutritivnih komponenata (krmiva) koja će zadovoljiti ishrambene standarde životinja za hranjivim metrijama (DeVries i sar., 2007). Korištenje TMR obroka može značajno stimulisati konzumaciju suhe materije do 5% (Linn, 1995). Komercijalni obroci uglavnom su sastavljani od voluminoznih krmiva u formi sijena, silaža i sjenaža i sl., uz redovnu suplementaciju koncentratnim (energetskim i proteinskim) krmivima i mineralnim, vitaminskim i drugim aditivima. Preciznija formulacija obroka optimizira odnos hranjivih materija, koji će kako u fizičkom tako i hemijskom smislu trebaju biti usklađena sa potrebama (normativima) proizvodnje (Coppock i sar., 1981). To znači da životinja konzumacijom svakog zalogaja unosi uravnoteženu količinu hranjivih materija (Beigh i sar., 2017). TMR obroci se pripremaju u specijalnim mikser prikolicama značajno olakšavajći pripremu hrane (usitnjavanje i homogenizacija) uz minimalnu upotrebu ljudske snage. Potreba za TMR obrokom javila se zbog uniformnosti i usklađenosti probavnih procesa bez značajnijih fluktuacija, ali i ograničenim mogućnostima životinja da biraju ukusnije dijelove obroka, a uglavnom na štetu voluminoznog dijela obroka. Ishrana kompletno miješanim obrocima primjenjuje se na većim farmama zbog opravdanosti i lakše organizacije (grupisanje životinja). Ovaj način ishrane ograničava i mogućnost selektiranja dijelova obroka. Bez obzira na sve, ipak se dešava da životinje izbjegnu konzumaciju krupnijih dijelova voluminoznih krmiva zbog neodgovarajućeg kvaliteta, predpripreme usjeva i nedovoljne usitnjenosti. Fizička efektivnost vlakana TMR-a je veoma važna pri formulaciji, a fizičke karakteristike vlakana u obroku imaju benefite na aktivnost žvakanja, buražnu fermentaciju, puferizaciju sadržaja buraga i na sadržaj mliječne masti (Stojanović i sar., 2011). Na fizičke karakteristike obroka utiče vrsta i udio voluminoznog i koncentratnog obroka i distribucija veličina čestica obroka. Vlakna predstavljaju sporije ili teže probavljive ili neprobavljive hranjive materije krmiva, koji zauzimaju značajnu zapreminu u probavnom sistemu preživara (Stojanović i sar., 2016). Prema Mertens (1997) koncept fizički efektivnih vlakana (peNDF) povezan je sa fizičkom efektivnošću (pef) koja se referira na prosječnu dužinu čestica koje podstiču preživanje, a utiču na aktivnost žvakanja i dvofaznu prirodu ruminalnog sadržaja (krupnije plutajuće čestice i tečna faza sitnijih čestica). peNDF je udruženi parametar nastao kada se sadržaj neutralanih deterdžentskih vlakana (NDF) pomnoži sa njegovom fizičkom efektivnošću. Povećan sadržaj peNDF stimulira aktivnost žvakanja i preživanja. Cilj istraživanja je utvrditi stepen selekcije (sortiranja) pojedinačnih frakcija TMR obroka u četiri vremenska intervala u vrijeme jutarnje ishrane i dinamiku konzumacije pripadajućih hranjivih materija i energije, odnosno sirovog proteina, škroba, kiselih deterdžentskih vlakana (ADF) i neutralnih deterdženstkih vlakana.

MATERIJAL I METODE

Istraživanje je provedeno na komercijalnoj farmi mliječnih krava „Spreča“ d.o.o. Kalesija (44°26´29˝N; 18°45´02˝E, 234 m.n.v.), čija je proizvodnja zasnovana na savremenoj tehnologiji. Kao materijal za ispitivanje korišten je kompletno miješani obrok (TMR) namijenjen za ishranu visokoproduktivnih krava Holštajn – frizijske pasmine koje su se nalazile u srednjem periodu laktacije. Eksperimentalne životinja su se nalazile u slobodnom sistemu ishrane i napajanja. Sve životinje su bile u dobrom zdravstvenom stanju i tjelesnoj kondiciji. Kompletno miješani obroci (TMR) su pripremani u miks prikolici u vremenskom intervalu od cca. 5 minuta, te servirani trokratno tokom dana. Kompletno miješani obrok je bio sastavljen od livadskog sijena, kukuruzne silaže, travne silaže i tri koncentratne smjese. Struktura i postotno učešće pojedinih komponenata (krmiva) prikazani su u tabeli 1. Dominantnu komponentu obroka činila je kukuruzna silaža sa udjelom od 40% na bazi suhe materije (SM). Procentualno učešće voluminoznog dijela obroka iznosio je 57,8%, dok je koncentratni dio obroka iznosio 42,2% na bazi suhe materije.

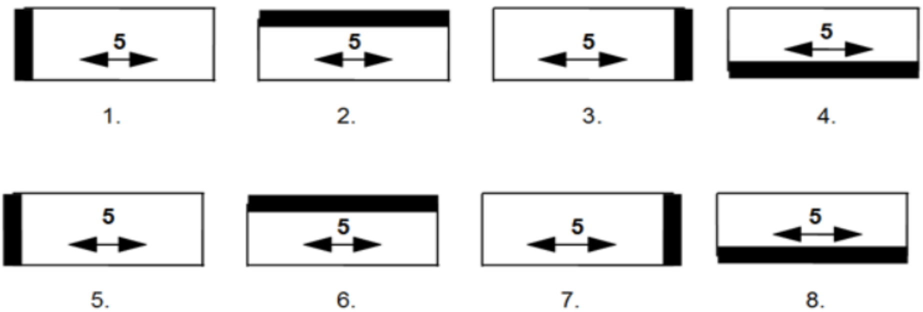
Tabela 1. Sastav kompletno miješanog obroka za ishranu laktirajućih krava u srednjoj fazi laktacije
Table 1. Composition of the total mixed ration for feeding lactating cows during the middle stage of lactation

Komponente kompletnog obroka *Total mixed ration ingredients*	**Originalno krmivo** *As fed basis*		**Suha materija krmiva** *Dry matter basis*	
	kg	%	kg	%
Livadsko sijeno *Medow grass hay*	3,0	7,4	2,8	14,4
Kukuruzna silaža *Corn silage*	30,0	61,7	7,9	39,9
Travna silaža *Haylage*	3,0	7,4	0,7	3,5
Koncentratna smjesa 1 *Concentrate mix 3*	4,0	9,9	3,5	17,7
Koncentratna smjesa 2 *Concentrate mix 2*	4,0	9,9	3,5	17,8
Koncentratna smjesa 3 *Concentrate mix 3*	1,5	3,7	1,4	6,8
Voluminozni dio *Forage ratio*	36,0	76,5	11,4	57,8
Koncentratni dio *Concentrate ratio*	9,5	23,5	8,3	42,2
Ukupno *Total*	45,5	100	19,7	100,0

Ukupan sadržaj suhe materije u obroku iznosio je 19,7 kg, odnosno 43,3% u originalnom uzorku. Uzorakovanje je vršeno sa krmnog stola neposredno nakon serviranja, a zatim sukcesivno nakon drugog, četvrtog i šestog sata (ISO 6496:1999). Dobro sabijeni i konzervisani ($HgCl_2$) uzorci su čuvani u staklenim teglama (2500 ml) u zamrzivaču na -25°C do momenta analiziranja. Svi uzorci su prethodno osušeni u sušnici (Memmert S25) na 55°C, a zatim usitnjeni mlinom (Tecator Cyclotec 1093) na prosječnu dužinu ≈ 1,0 mm (ISO 6498:2012). Određivanje sadržaja hemijskih parametara utvrđeni su prema referentnim metodama i to za sadržaj suhe materije i vlage (ISO 6496:1999), sirovog pepela (ISO 5984:2002), sirovog proteina (ISO 5983-1:2005; ISO 5983-2:2005), neutralnih deterdžentskih vlakana (ISO 16472:2003), kiselih deterdžentskih vlakana (ISO 13906:2008), škroba (ISO 6490:2000), nevlaknastih ugljikohidrata (računski), ukupno probavljive hranjive materije (TDN) i neto energije za laktaciju (NEL) prema Weiss (1998). Na paralelnom uzorku analizom prosijavanja utvrđena je prosječna veličina i distribucija čestica voluminoznih krmiva i potpuno kompletnih obroka (u originalnom sadržaju SM). Za tu svrhu korištena su Penn State Partical Separator (PSPS) sita (Modifikovani model ver. 2013) sa 4 milimetarskim donjim sitom, prema preporukama proizvođača (Pennsylvania State University). PSPS je namjenski granularni uređaj konstruisan za odvajanje (separaciju) veličina čestica voluminoznih krmiva i kompletnog obroka. Dobijeni rezultati su izraženi kao prosjek tri uzastopna mjerenja (prosijavanja). Računskim obrascima iz tabele 3, izračunat je procentualni udio pojedinih frakcija čestica na bazi suhe materije u svježem uzorku. Faktor fizičke efektivnosti (pef) je izračunat kao maseni zbir čestica obroka zadržanih iznad srednjeg (8 mm) i donjeg (4 mm) sita. Izračunate vrijednosti su iskorištene za kalkulaciju fizički efektivnih vlakana (peNDF) zadržanih iznad perforiranih sitia promjera rupa 8 i 4 mm, prema sljedećim matematičkim obrascima 1 i 2 (Lammers i sar. 1996; Kononoff i sar. 2003; Heinrichs, 2013):

(1) peNDF8mm = pef8mm x NDF%
(2) peNDF4mm = pef4mm x NDF%

Površina ekrana napravljena je od perforirane ploče sa okruglim porama. Sastavljena su od četiri plastična sita, poredana u nizu od gornjeg sita sa najvećim promjerom rupa (19 mm), srednjeg sita (8 mm), donjeg sita (4 mm) i ravnog dana. Sama izrada i tehničke specifikacije kombinovanih sita izrađena je prema projekciji stratifikacije hrane u buragu. Prva veličina ili gornje sito (19 mm) uglavnom zadržava čestice koje stimulišu preživanje i lučenje pljuvačke (gruba, čvrsta faza). Naredno ili srednje sito (8 mm) uglavnom zadržava čestice hrane koje formiraju čvrstu fazu, dok sito 4 mm zadržava vlaknaste i nevlaknaste čestice hrane koje se lahko degradiraju. Čestice manje od 4 mm završavaju na dnu sita i dio su tečne faze buraga koja je lahko probavljiva i koja prelazi u daljnju fazu hemijske digestije. Čestice zadržane na dnu i donjem sizu (4 mm) ne stimulišu pojavu preživanja. Na gornje sito se smjesti približno 1,4 do 1,5 litara reprezentativnog uzorka (Lammers i sar., 1996). Tehnika prosijavanja zasniva se na horizontalnom pomjeranju sita 5 puta u jednom smjeru (1 sekvenca). Proces završava sa osam sekvenci ili ukupno 40 pomjeranja, rotirajući sita nakon svake sekvence od 5 pokreta. Ilustrativan prikaz postupka prosijavanja prikazan je na sljedećoj shemi (Lammers i sar., 1996).



Slika 1. Prikaz tehnike prosijavanja na PSPS sitima
Figure 1. Illustration of the sieving technique using PSPS sieves

Za najbolje rezultate potrebno je primijeniti odgovarajuću frekvenciju pokreta prosijavanja od 1,1 Hz (otprilike 1,0 do 1,1 pokret/s), sa dužinom pokreta od 17 do 20 cm. Nakon završenog prosijavanja, potrebno je na tehničkoj vagi izvagati udio ukupnog uzorka zadržanog na svakoj pojedinačnoj površini sita i procentualno izraziti u odnosu na inicijalnu (početnu) masu (Tabela 2 i 3).

Tabela 2. Preporučena raspodjela veličine čestica voluminoznih krmiva i kompletno miješanih obroka prema preporukama Heinrichs (2013)
Table 2. Particle size distribution of forages and total mixed rations according to the recommendations of Heinrichs (2013)

Ekran *Screen*	Prečnik pore *Pore size* mm	Veličina čestica *Particle size* mm	Silaža *Silage* %	Sjenaža *Haylage* %	*TMR* %
Gornje sito *Upper sieve*	19,0	>19	3-8	10-20	2-8
Srednje sito *Middle sieve*	8,0	8-19	45-65	45-75	30-50
Donje sito *Lower sieve*	4,0	4-8	20-30	30-40	10-20
Dno *Bottom*	4,0	≤4	≤10	≤10	30-40

TMR – kompletno miješani obrok/total mixed ration

Tabela 3. Izračunavanje udjela pojedinih frakcija u uzorku i kumulativa (sume) procenta uzorka ispod navedenog sita
Table 3. Ratio calculation of individual fractions in the sample and the cumulative (sum) percentage of the sample below the specified sieve

Ekran *Screen*	Masa uzorka, g *Sample weight*	Udio frakcije, % *Fraction ratio*	Suma procenta ispod sita, %
Gornje sito *Upper sieve*	A	$X = A/E \times 100$	$F = 100 - (A/E \times 100)$
Srednje sito *Middle sieve*	B	$X = B/E \times 100$	$G = F - (B/E \times 100)$
Donje sito *Lower sieve*	C	$X = C/E \times 100$	$H = G - (C/E \times 100)$
Dno *Bottom*	D	$X = D/E \times 100$	0
Ukupna masa *Total weight*	E		

Za statističku obradu podataka korišten je IBM SPSS Statistics, statistički softverski paket (Windows *ver* 20). Rezultati uzoraka TMR obroka obrađeni su deskriptivnom statistikom i prikazani kao srednja vrijednost (aritmetička sredina) i standardna devijacija (±SD). Za testiranje statističke značajnosti razlika između dvije aritmetičke sredine korištena je jednofaktorijalna analiza varijanse i Fischerov LSD (Least significant difference) test.

REZULTATI I DISKUSIJA

Komercijalni TMR obrok se sastojao od uobičajenih krmiva za intenzivnu ishranu krava. U tabeli 4 su prikazane ishrambene vrijednosti krmiva i kompletno miješanog obroka (TMR). Relativno niži sadržaj SM u obroku ukazuje na značajnije učešće konzerviranih vlažnih krmiva poput kukuruzne silaže i travne sjenaže sa niskim sadržajem suhe materije (31,5% i 22,8%). Ovo potencijalno može biti ishrambeni problem za obrok jer može depresivno uticati na konzumaciju suhe materije (KSM). Najveći dio energetskog i proteinskog potencijala (sadržaj proteina i škroba), životinja podmiruje iz koncentratnih krmiva odnosno smjesa 1, 2, i 3, dok suha i vlažna konzrvirana krmiva značajno doprinose sadržaju vlakana i njenih frakcija. Svojim fizikalnim svojstvima značajno doprinose voluminoznošću obroka (tabela 1), te samim tim pozitivno utiču na fiziološke procese preživanja i puferizacije (Stojanović i sar., 2011). Ovakvom strukturom, obrok u najvećoj mjeri podmiruje sve potrebe za ispitivanu kategoriju i proizvodni status mliječnih krava, uz manja odstupanja poredeći ih sa preporukama (NRC, 2001).

Tabela 4. Hemijski sastav i energetska vrijednost krmiva i kompletno miješanog obroka (na bazi SM)
Table 4. Chemical composition and energy value of feeds and total mixed ration (DM basis)

Pokazatelj *Parameter*	LS	KS	TS	KS1	KS2	KS3	TMR
Suha metrija, % *Dry matter*	94,5	31,5	22,8	87,0	87,6	89,1	46,2
Sirove bjelančevine, % *Crude protein*	5,4	7,2	6,6	22,6	25,5	41,8	14,7
Neutralna deterdženstka vlakna, % *Neutral detergent fiber*	58,1	41,2	68,4	12,8	14,3	14,7	36,0
Kisela deterdženstka vlakna, % *Acid detergent fiber*	38,0	26,0	42,8	5,7	7,2	7,5	17,5
Škrob, % *Starch*	-	28,3	-	35,2	34,5	8,0	29,1
Neto energija laktacije, MJ/kg SM *Net energy for lactation*	4,8	6,8	4,1	8,3	8,2	8,1	6,0

LS – livadno sijeno/grass hay; KS – kukuruzna silaža/corn silage; TS – travna silaža/haylage; KS1,2,3 – koncentratna smjesa 1,2,3/concentrate mixture 1,2,3; TMR – kompletno miješani obrok/total mixed ration, SM – suha materija/dry matter

Analiza fizikalnih svojstava TMR obroka je novija dopuna ishrambenoj formulaciji. Kada su u pitanju fizikalne karakteristike, analize su pokazale da se radi o obroku koji u najvećoj mjeri zadovoljava kvantitavne osobine distribucije čestica, sa nešto nižom vrijednosću prosječne veličine čestice (5,3 mm), što ga svrstava u nešto sitnije pripremljene obroke, sa potencijalno (rizičnim) negativnim implikacijama na probavne i proizvodne performase. Rezultati fizikalnih analiza TMR obroka generalno odgovaraju zahtjevima, odnosno 3,4%, 32,5%, 28,6% i 35,5% za sva četiri sita ulaze u referentne rangove prema Heinrichs (2013). Pravi efekti konzumacije ovakvog obroka bi se mogli sagledati posmatranjem pH vrijednosti buražnog sadržaja i sadržaja masti u mlijeku. Posmatrano kroz različite vremenske tretmane koje su prikazane u tabeli 5, fizikalne karekteristike pokazuju značajna statistička odstupanja kod svih ispitivanih parametara. Mjerenja u kasnijim intervalima neravnomjerno naglašavaju vrijednosti u prva dva, dok su vrijednosti za donja dva sita značajno reducirana. Pa je tako sadržaj veličine čestica rastao od 5,34 do 7,97 mm, frakcije >19mm 3,36% do 16,20%, i 8 mm od 32,53% do 38,97%, dok kod 4 mm frakcije opadao od 28,60% do 22,53% i <4 mm od 35,51% do 22,30%.

Tabela 5. Prosječna veličina čestica (mm) i raspodjela kompletno miješanog obroka obroka prema vremenskim intervalima izražena u postotcima (x±σ)
Table 5. Average particle length and distribution of total mixed ration according to feeding intervals (x±σ)

Pokazatelj *Parameter*	Period nakon ishrane, sati *Feeding period after, hours*				P^*
	0.	2.	4.	6.	
Veličina čestice, mm *Particle size*	5,34[a] ±0,37	5,74[a] ±0,34	6,64[b] ±0,86	7,97[c] ±0,46	0,000
Gornje sito, % *Upper sieve*	3,36[a] ±0,90	4,28[a] ±1,10	12,46[b] ±1,43	16,20[c] ±3,07	0,000
Srednje sito, % *Middle sieve*	32,53[a] ±3,25	37,31[b] ±2,13	38,59[b] ±1,20	38,97[b] ±0,56	0,017
Donje sito, % *Lower sieve*	28,60[a] ±1,78	24,90[b] ±0,95	23,80[b] ±0,46	22,53[b] ±1,71	0,003
Dno, % *Bottom*	35,51[a] ±3,39	33,52[ab] ±2,34	27,48[bc] ±5,70	22,30[c] ±1,00	0,007

[*] nivo značajnosti 0,05/significance level at 0.05; [a,b,c,d] različita slova u istom redu označavaju različite sredine tretmana/different letters in the same row denote different treatment means; x – aritimetička sredina/arithmetic mean; σ – standardna devijacija/standard deviation

Uvidom u sadržaj gornjeg i srednjeg sita uglavnom se radi o dužim česticama voluminoznog dijela obroka u kojima dominira livadno sijeno i travna sjenaža sa primjesama krupnijih dijelova kukuruzne silaže. Srednje sito uglavnom sadrži manje čestice kukuruzne silaže (zajedno sa nagnječenim zrnom), dok dno uglavnom sadrži sitnije čestice koncentratnih smjesa. Ovakva raspodjela učešća pojedinih frakcija obroka kroz vremenske tretmane ukazuje na neravnomjernu konzumaciju pojedinačnih frakcija TMR obroka tj. pojavu selektivnog ishrambenog ponašanja životinja koje ciljano preferiraju sitnije, ukusnije i mekše dijelove uglavnom koncentratnog dijela obroka (Leonardi i sar., 2005; Kononoff i sar., 2003). Međutim, mnogo tačnije procjene o efektima fizičkih svojstava dokazuju se pokazateljima fizičke efektivnosti vlakana (peNDF). U tabeli 6 su prikazane vrijednosti navedenih parametara i one se kroz tretmane kreću od 13,0% do 26,7% za $peNDF_{8mm}$ i 23,3% do 37,6% za $peNDF_{4mm}$. Vrlo slične rezultate distribucije pojedinih frakcija kod kompletnih obroka su u svom istraživanju utvrdili Stojković i sar. (2021), Hadžić (2020); Simoni i sar. (2021), Damery i sar. (2017) i dr. Za održavanje optimalne pH vrijednosti buraga (pH ≥ 6) i mliječne masti (3,4%), sadržaj $peNDF_{4mm}$ treba da iznosi najmanje 20% SM, pogotovo ako se radi o visokokoncentratnim obrocima sa 50 do 60% SM (Mertens, 1997).

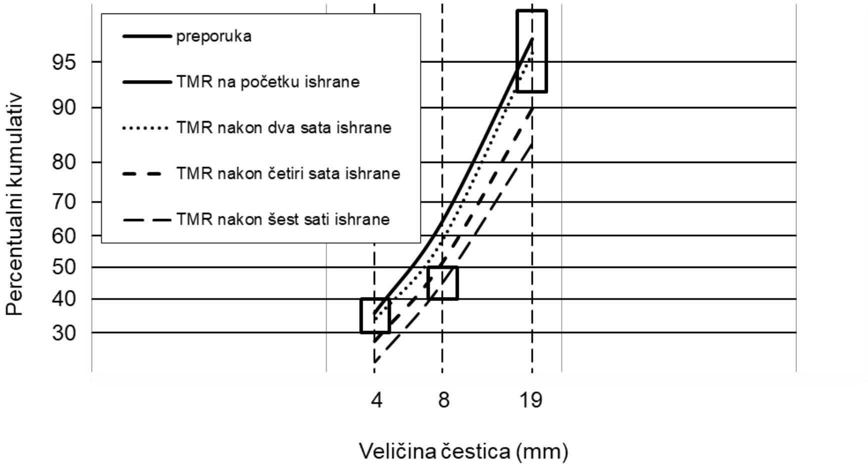
Tabela 6. Rezultati fizičke efektivnosti i efektivnih vlakana kompletno miješanih obroka kroz različite intervale ishrane (x±σ).

Table 6. Results of physical effectiveness and effective fiber for total mixed rations through different feeding intervals (x±σ)

Pokazatelj *Parameter*	Period nakon ishrane, sati *Feeding period after, hours*				P^*
	0.	2.	4.	6.	
Pef_{8mm}	0,359^a ±0,04	0,412^a ±0,03	0,476^b ±0,05	0,537^c ±0,04	0,000
Pef_{4mm}	0,645^a ±0,03	0,663^a ±0,02	0,726^b ±0,04	0,770^c ±0,02	0,000
NDF, %	36,03^a ±2,26	38,40^{ab} ±1,63	41,77^b ±2,14	48,37^c ±0,91	0,000
$peNDF_{8mm}$, %	12,92^a ±1,39	15,82^b ±1,17	19,87^c ±2,01	25,99^d ±1,77	0,000
$peNDF_{4mm}$, %	23,25^a ±1,23	25,47^b ±0,79	30,32^c ±1,58	37,23^d ±0,77	0,000

* nivo značajnosti 0,05/significance level at 0.05; ^{a,b,c,d} različita slova u istom redu označavaju različite sredine tretmana/different letters in the same row denote different treatment means; x – aritimetička sredina/arithmetic mean; σ – standardna devijacija/standard deviation; pef – faktor fizičke efektivnosti kao udio častica zaostala na 2 i 3 sita/physical effectiveness factor as the proportion of particles retained on 2 and 3 sieves; NDF – neutralna deterdžentska vlakna/neutral detergent fiber; peNDF – fizički efektivan NDF/physically effective NDF

Poredeći sa rezultatima ovog istraživanja može se zaključiti da TMR obroci dovoljno stimulišu aktivnost žvakanja i produkciju pljuvačke. Zebeli i sar. (2010) preporučuju da se vrijednosti $peNDF_{8mm}$ za srednju i kasnu laktaciju trebaju iznositi minimalno 10,9-18,5%. Kad je u pitanju efektivnost vlakana na nivou 4 mm prema Mertens (1997) trebala bi iznositi minimalno 20%, a optimalno 22,3%. Vrijednosti $peNDF_{4mm}$ su se kretale od 12,92% do 25,99%. Respektabilan broj studija pokazao je da je održavanje sadržaja NDF na nivou između 30% i 32% dovoljan da osigura sadržaj mliječne masti od 3,4%, bez obzira na izvor (porijeklo) škroba (Mertens, 1997; Kononoff i Heinrichs, 2003, Silveira i sar., 2007). Ovo istraživanje je pokazalo, a ujedno i potvrdilo da fizičko – hemijske karakteristike obroka prikazanog u tabeli 6 mogu održati koncentraciju mliječne masti značajno iznad 3,4%, obzirom da se u obroku sadržaj NDF-a kretao 36,03% na početku, do 48,37% na kraju ispitivanog perioda ishrane. Na grafikonu 1 se jasno vidi da postoje značajnija odstupanja fizikalnih karakteristika obroka kako vrijeme ishrane odmiče.



Grafikon 1. Odstupanja distribucije i veličine čestica kompletno miješanih obroka kroz vremenske intervale od preporučenih vrijednosti
Graph 1. Deviations of the distribution and particle size of completely mixed meals over time intervals compared to the recommended values

Prema DeVries i sar. (2007) i Hosseinkhani i sar. (2008) sortiranje smanjuje nutritivnu vrijednost TMR obroka, što je posebno izraženo nakon nekoliko sati nakon serviranja, jer može predstavljati problem socijalno inferiornijim kravama u slobodnom sistemu držanja. Efekat selektivnosti se pojačava kod krava hranjenih obrocima sa većim sadržajem SM (>55%) iz voluminoznog dijela obroka i pod snažnim je uticajem fiziološkog stanja i faze laktacije (Leonardi i sar., 2005; DeVries i sar., 2007). Dosadašnja istraživanja o važnosti procjene efektivnosti mnogo je značajnija kod ishrane krava u ranoj fazi laktacije sa značajnijim udjelom (>50%) koncentratnim krmiva u obroku (Llonch i sar., 2020). Sadržaj suhe materije i sredina laktacije u ovom istraživanju ne ide u prilog ovakvoj konstataciji.

Tabela 7. Hemijski sastav i energetska vrijednost frakcija kompletno miješanih obroka po intervalima ishrane (na bazi SM; x±σ)
Table 7. Chemical composition and energy values of total mixed ration fractions by feeding intervals expressed (DM basis; x±σ)

Pokazatelj *Parametes*	Period nakon ishrane, sati *Feeding period after, hours*				P^*
	0.	2.	4.	6.	
Suha materija, % *Dry matter*	46,20[a] ±1,85	45,80[a] ±2,15	41,97[b] ±1,32	41,20[b]± 0,70	0,010

Sirove bjelančevine, % *Crude protein*	14,66[a] ±0,25	13,32[b] ±0,06	12,48[c] ±0,27	11,71[d]± 0,23	0,004
Neutralna deterdžentska vlakna, % *Neutral detergent fiber*	36,03[a] ±2,26	38,40[ab]± 1,63	41,77[b] ±2,14	48,37[c]± 0,91	0,000
Kisela deterdžentska vlakna, % *Acid detergent fiber*	17,51[a] ±1,16	18,38[a] ±1,07	20,30[b] ±0,93	22,74[c]± 0,56	0,001
Škrob, % *Starch*	29,05[a] ±0,75	27,53[b] ±0,66	25,27[c] ±0,36	22,32[d]± 1,10	0,000
Neto energija laktacije, MJ/kg *Net energy for lactation*	5,98[a] ±0,08	5,89[ab] ±0,07	5,78[b] ±0,09	5,52[c] ±0,03	0,000

[*] nivo značajnosti 0,05/significance level at 0.05; x – aritimetička sredina/arithmetic mean; σ – standardna devijacija/standard deviation; [a,b,c,d] različita slova u istom redu označavaju različite sredine tretmana/different letters in the same row denote different treatment means

U tabeli 7 su prikazani rezultati fizičko-hemijskih analiza TMR obroka kroz vremenske intervale ispitivanja. Obzirom da su uzorci uzorkovani svaka dva sata, sadržaj suhe materije i hranjivih materija se signigikantno mijenjao (P=0,000*)*. Sadržaj suhe materije se kretao od 46,20% na početku do 41,20% kod zadnjeg mjerenja. Primjetan pad sadržaja suhe materije u TMR može biti posljedica sortiranja i selekcije suhljih čestica koncentratnih krmiva koje imaju visok sadržaj suhe materije (≈ 88%). Ovaj trend se može potkrijepiti descedentnim kretanjem sadržaja sirovog proteina (14,44-11,71%) i skroba (29,05-22,32%), te rastom sadržaja neutralnih (36,03-48,37%) i kiselih (17,51-22,74%) deterdžentskih vlakana. Dodatni, ali minorni efekat može biti i kontinuirano vlaženje TMR smjese pljuvačkom tokom perioda ishrane krava. U svakom od navedenih intervala posmatranja sadržaj suhe materije u TMR smjesama nalazi se na donjoj granici ili je nešto niži od optimalnih vrijdenosti (45-55%). Obzirom na preporuke konzumacije suhe materije (KSM), obrok prikazan u tabeli 4 i 7 ne sadrži dovoljne količine suhe materije, iako teoretski može odstupati do 10% od preporučenog. Tome u prilog pokazuje i nešto niži sadržaj neto energije obroka (6,0-5,5 MJ/kg). NRC preporučuje da koncentracija energije u obroku bude oko 7 MJ/kg.

Tabela 8. Hemijski sastav i energetska vrijednost TMR obroka na različitim promjerima PSPS sita (x±σ)

Table 8. Chemical composition and energy value of TMR on different PSPS sieve diameters (x±σ)

Pokazatelj *Parameter*	>19 mm	8-19 mm	4-8 mm	<4 mm	*P*[*]
Suha materija, % *Dry matter*	39,73[a]± 2,47	40,34[a]± 1,82	41,29[a]± 2,25	45,88[b]± 1,04	0,003
Sirove bjelančevine, % *Crude protein*	9,46[a] ±0,77	9,51[a] ±0,57	11,40[b]± 0,75	16,94[c]± 0,75	0,000
Neutralna deterđentska vlakna, % *Neutral detergent fiber*	54,94[a]± 3,30	45,86[b]± 3,20	40,74[c]± 2,50	32,89[d]± 2,21	0,000
Kisela deterdžentska vlakna, % *Acid detergent fiber*	30,84[a]± 2,07	21,38[b]± 1,29	19,58[c]± 1,05	12,57[d]± 0,54	0,000

Škrob, % *Starch*	9,24[a] ±3,00	22,07[b]± 2,27	24,47[c]± 1,78	36,12[d]± 1,72	0,000
Neto energija laktacije, MJ/kg *Net energy for lactation*	5,59[a] ±0,19	5,88[b] ±0,21	6,01[bc] ±0,23	6,99[d] ±0,27	0,000

[*] nivo značajnosti 0,05/significance level at 0.05; [a,b,c,d] različita slova u istom redu označavaju različite sredine tretmana/different letters in the same row denote different treatment means; x – aritimetička sredina/arithmetic mean; σ – standardna devijacija/standard deviation

Također u tabeli 8 su prikazane vrijednosti fizičko-hemijskih parametara pojedinačnih frakcija, na osnovu koje se može vidjeti da značajan udio suhe materije, sirovog proteina i škroba je sadržan u frakcijama na donjem situ i dnu sita kao najsitnije frakcije. S druge strane krupnije frakcije čestica TMR obroka koje su zadržane na srednjem i gornjem situ su bogatije neutralnim i kiselim deterdžentskim vlakanim, a koje značajno utiču na fizičku efektivnost obroka. Ekstremno nizak unos može ukazivati na promjenu kvaliteta krmiva ili strukture obroka. Ključ za pravilno formulisanje kompletno miješanog obroka je optimizacija konzumacije suhe materije (Heinrichs i Kmicikewycz, 2016). Preferencijalno konzumiranjem koncentratnih krmiva iz obroka pokazalo je opadanje sadržaj sirovog proteina i škroba nakon svakog drugog sata uzorkovanja, što je uticalo na smanjenje sadržaj suhe materije u ostatku obroka. Sadržaj sirovog proteina iz TMR-a na početku konzumiranja obroka iznosio je 14,67%, dok je šesti sat nakon hranjenja iznosio 11,71%. Eksperimentalni obrok je sadržavao više NDF-a i ADF-a, kao djelimično probavljivih materija. Prema *NRC* preporukama za mliječne krave iz 2001. (Linn, 2003), nutritivna vrijednost TMR obroka za srednju fazu laktacije skoro pa odgovara preporučenim vrijednostima. Naime, sadržaj sirovog proteina u obroku za krave u srednjoj fazi laktacije je nešto niži, sadržaj NDF je za 3% viši, ADF nešto manji, dok je sadržaj škroba za 2% nešto viši od preporučenih vrijednosti. U konačnici, nutritivna vrijednost TMR obroka korištenih u ovom radu, značajno ne odstupaju od NRC preporuka. Tabela 8 pokazuje trend kretanja sadržaja pojedinih hranjivih materije u esprimentalnom obroku kroz ispitivane intervale. Škrob je važna komponenata TMR-a i uglavnom vodi porijeklo iz koncentratnih smjesa i kukuruzne silaže, a imao je opadajući trend kroz vremenske intervale od 29,05% do 22,33%. S obzirom da je značajan dio neto energije sadržan u škrobu, pozitivno korelira sa rezultatina neto energije. Sadržaj suhe materije u dijelu obroka na gornjem situ (>19 mm) iznosila je 39,73%, dok se smanjenjem veličine čestica povećavala i na dnu sita iznosila 45,88%. Veći sadržaj SM u nižim frakcijama obroka i njihova značajnija konzumacija je glavni razlog opadanja sadržaja SM u TMR obroku kroz ispitivane intervale. Obzirom da frakcija TMR-a koja ostaje na dnu sita sadrži sitne čestice obroka, odnosno u najvećoj mjeri koncentrate, kao rezultat se javlja povećana konzumacija proteina. Sadržaj sirovog proteina na gornjem situ (>19 mm) iznosio je 9,85%, dok je donja najsitnija frakcija sadržavala 17,53% SP, tj. sa smanjenjem veličine čestica, sadržaj sirovog proteina je rastao. NDF i ADF vlakna kao djelimično probavljiva ili neprobavljiva komponenta obroka najviše su se akumulirale na gornjem i srednjem situ, te se sa smanjenjem veličine čestica i njihova koncentracija smanjuje. Sadržaj neto energije najviše je zastupljen u frakcijama zaostale na donjem i dnu sita i iznose 6,01 MJ/kg SM odnosno 6,99 MJ/kg SM, jer se radi uglavnom o frakcijama koje sadrže lahko probavljivi škrob porijeklom iz koncentratih krmiva (Tabela 8).

ZAKLJUČAK

Istraživanjem su utvrđene značajne statističke razlike između konzumirane hrane kroz ispitivane vremenske intervale, po svim ispitivanim hemijskim i parametrima fizičke efektivnosti. Ovakvi rezultati navode na zaključak o umjerenoj pojavi preferencijalnog sortiranja TMR obroka. Analizom strukture obroka dokazan je voluminozni karakter potpuno kompletnog obroka (>50%), što je karakteristično za ishranu krava u srednjoj fazi laktacije. Na početku ishrane, krave su u najvećoj mjeri konzumirale sitnije, mekše i ukusnije frakcije TMR iz koncentratnih smijesa, a zatim krupnije, grublje čestice voluminoznog dijela obroka pri kraju posmatranog perioda. Na osnovu povećanja sadržaja vlaknastih komponenata obroka kroz ishrambeni period, na šta ukazuju i parametri pef i peNDF, može se pretpostaviti o značajnijoj stimulaciji žvakanja i preživanja što je krucijalno za održavanje normalne buražne fermentacije. Utvrđena prosječna veličina čestica, svrstala je obrok u fino mljeveniji što nije poželjna forma pripreme.

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THE EFFECT OF PHYSICAL EFFICIENCY OF FIBER ON THE CONSUMPTION AND SELECTION OF COMPONENTS OF TMR RATION FOR LACTATING COWS

Abstract

Total mixed ration (TMR) consists of many different nutrients contained in feeds of various origins, of a certain amount and composition, mixed into one meal. This structure of the meal began to be used due to the ability of animals to choose parts of the meal during feeding, preferring smaller and more digestible particles. By using TMR meals, animals are limited in their ability to select food, especially hay and silage, which is very important in the process of digestion and breakdown of food. Physically effective fibers are important for the prevention of various health disorders, chewing activity, rumen fermentation, pH value of rumen content, and fat content in milk. To determine the degree of selection of individual fractions of the TMR ration through time intervals, it is necessary to carry out appropriate experimental tasks such as sampling and fractionation of the TMR ration and different chemical analyses of nutrients in the completely mixed ration. Based on the analysis, it can be determined how much the physical effectiveness of fibers affects the consumption and selection of certain components of the TMR. Although TMR is a complete meal, various analyses have determined that selectivity still occurs on the part of cows in the mid-lactation phase, which consumed the smallest, more digestible food particles to the greatest extent. The $peNDF_{8mm}$ content ranged from 12.92 to 25.99%, while the $peNDF_{4mm}$ content varied from 23.25 to 37.23% throughout the feeding period. Such a dynamics of nutrition showed that in the first hours of nutrition, the most concentrated part of the TMR meal is consumed, followed by hay and silage that encourages chewing and rumination of food. This way of consuming feeds is a consequence of uneven nutrition, after which there are changes in the conditions of digestion in the rumen. The analysis of the experimental TMR ration proved the voluminous character of the ration, which, based on the average length of the particles, was classified as a finely ground ration. In addition to the aforementioned analyses, for more precise assessments of the physical effectiveness of TMR rations, additional tests can be included, such as measuring the pH value in the rumen and measuring the content of various parameters of milk, especially milk fat.

Keywords: *total mixed ration (TMR), selection, physically effective fibers, physical effectiveness*

INTRODUCTION OF NEW FISH SPECIES IN BORAČKO LAKE OVER THE LAST 20 YEARS*

Dino Lepara[1], Samir Muhamedagić*[1]

Original scientific paper

Abstract

Boračko Lake is a natural mountain and glacial lake located at the base of the Prenj mountain, at an elevation of 397 meters. From the ichthyological aspect, this lake was originally of a salmonid character, inhabited exclusively by lake trout. However, over the past few decades, the ichthyofauna of this lake has been significantly changed due to the introduction of other fish species. Ichthyological research conducted at Boračko Lake in 2003, 2011 and 2018 revealed the presence of several new fish species, predominantly cyprinids (fish from the order Cypriniformes). Only a small number of individuals of salmonid fish species were registered. Ichthyological research conducted in 2023 confirmed the presence of several new fish species, which were not registered in earlier research. Today, 11 fish species live in Boračko Lake, among which the most numerous are cyprinids (7 species) and they make up 75.3% of the lake's total ichthyopopulation, while salmonids represent two species with a total share of 6.9%. The rest of the ichthyopopulation of this lake is made up of pike-perch with a share of 14.4%, while Neretva spined loach participates with a share of 3.4% of the total ichthyopopulation. In the coming period, a systematic approach is needed to address the issues through selective fishing of non-native (allochthonous) and invasive fish species in order to preserve, protect and restore the populations of indigenous species.

Keywords: *Boračko Lake; salmonids; cyprinids; introduction; research*

INTRODUCTION

The upper course of the Neretva River (geographically defined as the section stretching from its source to the confluence with the Rama River), also known locally as "Upper Neretva," exhibits all the characteristics of a true mountain river, with a gradient of approximately 15‰ (Drešković and Đug, 2010). In the upper Neretva area, a large number of mountain rivers and streams flow into the Neretva River, draining water from the high Bosnian and Herzegovinian mountains: Zelengora, Lebršnik, Živanj, Crvanj, Treskavica, Visočica, Bjelašnica and Prenj (Mihić, 1985; Vegara *et al.,* 2009;

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Muhamedagić, 2014). This area is also characterized by numerous mountain lakes, which are often referred to as "mountain eyes" by locals due to their beauty. Among the largest and most well-known lakes are Kotlaničko, Orlovačko, Jugovo, Crno, Bijelo, Donje bare, Gornje bare, Štirinsko and Kladopoljsko on Zelengora; Veliko, Platno, Crno and Bijelo on Treskavica; Blatačko Lake on Bjelašnica; Uloško Lake on Crvanj and Boračko Lake on Prenj (Sofradžija *et al.,* 2003; Vegara *et al.,* 2009; Drešković and Đug, 2010; Škrijelj *et al.,* 2011; Muhamedagić, 2014). At the foot of the northeastern slopes of mountain Prenj lies Boračko Lake, one of the largest and most famous mountain lakes in Bosnia and Herzegovina (Muhamedagić, 2014). Boračko Lake is oriented northwest-southeast, located on the eastern foothills of Prenj, at an altitude of 397 meters. The basin where the lake formed has a funnel-shaped, amphitheater-like appearance. The lake is surrounded by Crna gora (1,343-1,595 meters) to the southwest, Tranjina (1,055 meters) to the east, Košuta (490 meters) to the southeast, and the steep Krstac (approximately 750-800 meters above sea level) to the north, separating it from the village of Borci (Mihić, 1985; Škrijelj *et al.,* 2011). The surface area of Boračko Lake is 0.241 km^2, with a maximum depth of 14.0 meters (Drešković and Đug, 2010; Škrijelj *et al.,* 2011).

The rate at which non-native freshwater fishes have been introduced worldwide has doubled in the space of 30 years (Gozlan *et al.,* 2010). Modern ichthyological research started during the same period that Willi Hennig first proposed the approach of phylogenetic systematics for investigating biological questions, following Darwin's concept of natural classification (Chen and Mayden, 2010). The basis of all ichthyological research lies in studying the fish populations in the research area. The primary task of ichthyological research is to identify existing fish populations, ichthyological analyses and obtain relevant data on the presence of specific fish species in the studied locations. The results of ichthyological analyses also provide data on the status of fish populations, their structure and whether the population is in decline, stagnation or growth. Therefore, such research has both fundamental and applied importance for fisheries, as the ultimate aim is to protect the living world of that area, which will certainly be achieved by putting a certain aquatic ecosystem under control and bringing the exploitation of fish populations, i.e. fish stocks, within the limits of reasonable exploitation. The goal of these studies is to assess the structure of fish populations, as well as the distribution of ichthyopopulations in the investigated locations (Hamzić, 2003; Sofradžija *et al.,* 2003; Škrijelj *et al.,* 2011).

In the elaboration of the current state and projections of the protection and improvement of ichthyoresources in the waters of the Konjic municipality, information on the experiences of previous stocking with “indigenous” and non-native species is particularly important, especially when it comes to qualitative and quantitative indicators of these interventions and the origin (regional and population affiliation) of the introduced material. Without a planned and scientifically grounded approach to protecting and harmonizing appropriate food chains in local ecosystems, restrictions on fishing in selected parts of certain watercourses (bans, spawning grounds, etc.) and other complementary measures commonly implemented by sports fishing organizations to stimulate fish population growth may become self-serving, leading to futile investment of labor and resources. However, we are witnessing frequent unplanned and unprofessional stocking efforts, involving not only new species but also foreign fish species. This method of stocking is particularly vulnerable to indigenous salmonid fish populations, which occupy narrow habitats. Introduced non-native species, which are in many cases more resistant and aggressive, gradually displace indigenous salmonid populations, and in many cases, indigenous populations disappear entirely in such areas (Škrijelj *et al.,* 2011).

MATERIALS AND METHODS

Ichthyological research on the Boračko Lake ecosystem was based on fish samples caught with standard fishing tools, nets and an electrofishing generator. Stationary nets were used with a mesh diameter ranging from 10 to 36 mm. The length of the nets ranged from 25 to 50 m and their height from 1.20 to 6 m. They were tied in sets of 3 to 5 nets in order to cover multiple depth zones across the water flow. The sets of nets were set up in the evening and lifted up in the morning. In addition to the nets, a "Honda" FEG 15.000 (15 kW) electrofishing generator was used in the field. Fishing was conducted at three microlocations in the lake, so different depth zones were covered. The total investigated lake area was 1,500 m^2. The collected fish samples were processed in the field and returned to their natural habitat, while a smaller number of representative specimens were preserved in 4% formaldehyde and transported to the laboratory of the Center for Aquaculture and Fisheries at the Faculty of Agriculture and Food Sciences in Sarajevo, where they were further processed. During the catch, the fish specimens were properly preserved, labeled, stored at the appropriate temperature and delivered for laboratory analysis. When preparing the samples for laboratory examination, the delivered fish specimens were visually inspected, and after dissection, the internal organs were examined for the presence of appropriate clinical signs of bacterial and parasitic diseases. Systematic fish identification was conducted according to Vuković and Ivanović (1971): Ribe Bosne i Hercegovine; and Kottelat and Freyhof (2007): Handbook of European Freshwater Fishes.

RESULTS AND DISCUSSION

In the ichthyological research of Boračko Lake, which were conducted in 2003 as part of developing the fisheries baseline document (Sofradžija *et al.,* 2003), 7 fish species were registered: lake trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) from the Salmonidae family; and 5 species from the Cyprinidae family (common carp – *Cyprinus carpio*, Prussian carp – *Carassius gibelio*, chub – *Squalius cephalus*, Eurasian minnow – *Phoxinus phoxinus* and schneider – *Alburnoides bipunctatus*). The qualitative and quantitative composition of the ichthyopopulations of Boračko Lake is presented in Table 1.

Table 1. The qualitative and quantitative composition of the fish populations of Boračko Lake in 2003 (Sofradžija *et al.,* 2003)

Order	Family	Species	Abundance	
			Absolute (n)	Relative (%)
Salmoniformes	Salmonidae	Lake trout (*Salmo trutta*)	5	11.40
		Rainbow trout (*Oncorhynchus mykiss*)	3	6.80
Cypriniformes	Cyprinidae	Common carp (*Cyprinus carpio*)	11	25.0
		Prussian carp (*Carassius gibelio*)	5	11.40
		Chub (*Squalius cephalus*)	11	25.0
		Eurasian minnow (*Phoxinus phoxinus*)	1	2.20
		Schneider (*Alburnoides bipunctatus*)	8	18.20
Total		7	44	100.0

Analyzing the data from Table 1 shows a significantly higher abundance of fish belonging to the Cyprinidae family and a notably higher density of their populations. Specifically, common carp and chub make up 50.0% of the total abundance, while schneider, Prussian carp and Eurasian minnow together comprise 31.8%. The remaining 18.2% of the relative abundance consist of salmonid species, with lake trout making up 11.4% and rainbow trout 6.8%. The research has also shown an uneven distribution of certain fish species in different zones of the lake. The qualitative and quantitative structure of the ichthyofauna of Boračko Lake was analyzed on a total sample of 44 individuals (Sofradžija *et al.,* 2003).

According to the literature (Vuković, 1977; Vuković and Ivanović, 1971), Boračko Lake was inhabited exclusively by salmonid fish species, specifically the indigenous lake trout (*Salmo trutta* morpha *lacustris*) and the Ohrid trout (*Salmo letnica*), which was introduced from Ohrid Lake. Therefore, it can be concluded that by the end of the 20th century, at least 6 fish species, mostly cyprinids, had been introduced into Boračko Lake.

Based on the ichthyological research conducted on Boračko Lake in 2011 (Škrijelj *et al.,* 2011), ten fish species from four families of freshwater ichthyofauna (Salmonidae, Cyprinidae, Cobitidae and Percidae) were recorded. Three fish species were registered within the Salmonidae family: lake trout, Ohrid trout and peled (*Coregonus peled*); five species from the Cyprinidae family: common carp, Prussian carp, chub, Eurasian minnow and gudgeon (*Gobio gobio*); and one species each from the Cobitidae family (Neretva spined loach – *Cobitis narentana*) and from the Percidae family (pike-perch – *Sander lucioperca*). The qualitative and quantitative composition of the fish populations of Boračko Lake is shown in Table 2.

Table 2. The qualitative and quantitative composition of the fish populations of Boračko Lake in 2011 (Škrijelj *et al.,* 2011)

Order	Family	Species	Abundance	
			Absolute (n)	Relative (%)
Salmoniformes	Salmonidae	Lake trout (*Salmo trutta*)	9	4.10
		Ohrid trout (*Salmo letnica*)	8	3.70
		Peled (*Coregonus peled*)	1	0.50
Cypriniformes	Cyprinidae	Common carp (*Cyprinus carpio*)	49	22.40
		Prussian carp (*Carassius gibelio*)	36	16.40
		Chub (*Squalius cephalus*)	75	34.20
		Eurasian minnow (*Phoxinus phoxinus*)	22	10.0
		Gudgeon (*Gobio gobio*)	6	2.70
	Cobitidae	Neretva spined loach (*Cobitis narentana*)	12	5.50
Perciformes	Percidae	Pike-perch (*Sander lucioperca*)	1	0.50
Total		10	219	100.0

Based on Table 2, it can be concluded that the majority of the total ichthyopopulation (more than ¾) in Boračko Lake consist of fish from the Cyprinidae family, with a total share of 85.7% – chub is the most represented species with a share of 34.2%, common carp is represented with a share of 22.4%, Prussian carp with 16.4%, Eurasian minnow with 10.0%, while gudgeon is represented with a share of 2.7%. Salmonid fish species make up only 8.3% of the total share – lake trout is represented by 4.1%, Ohrid trout by 3.7%, while peled is represented by a share of 0.5%. The rest of the ichthyopopulation of Boračko Lake includes the Neretva spined loach (a representative of the Cobitidae family) with a total share of 5.5%, and pike-perch (a representative of the Percidae family) with a share of 0.5%. A total of 219 fish specimens were caught using electrofishing generator and nets (Škrijelj *et al.,* 2011).

Compared to the results of ichthyological research from 2003 (Sofradžija *et al.,* 2003), 4 new fish species have been recorded: peled, gudgeon, Neretva spined loach and pike-perch, while rainbow trout and schneider were not registered as they had been in the previous research.

The revision of the fisheries baseline document conducted in 2018 (Poljoprivredno-prehrambeni fakultet Sarajevo, 2018) established 9 fish species from six families of freshwater ichthyofauna (Salmonidae, Cyprinidae, Leuciscidae, Gobionidae, Cobitidae and Percidae). Three species from the Salmonidae family were registered: lake trout, Ohrid trout and peled; two species from the Cyprinidae family (common carp and Prussian carp); chub was registered from the Leuciscidae family; gudgeon was registered from the Gobionidae family; Neretva spined loach was registered from the Cobitidae family; while pike-perch was registered from the Percidae family. The qualitative and quantitative composition of the fish populations of Boračko Lake is presented in Table 3.

Table 3. The qualitative and quantitative composition of the fish populations of Boračko Lake in 2018 (Poljoprivredno-prehrambeni fakultet Sarajevo, 2018)

Order	Family	Species	Abundance	
			Absolute (n)	Relative (%)
Salmoniformes	Salmonidae	Lake trout (Salmo trutta)	6	6.90
		Ohrid trout (Salmo letnica)	4	4.60
		Peled (Coregonus peled)	1	1.10
Cypriniformes	Cyprinidae	Common carp (Cyprinus carpio)	12	13.80
		Prussian carp (Carassius gibelio)	12	13.80
	Leuciscidae	Chub (Squalius cephalus)	38	43.70
	Gobionidae	Gudgeon (Gobio gobio)	4	4.60
	Cobitidae	Neretva spined loach (Cobitis narentana)	6	6.90
Perciformes	Percidae	Pike-perch (Sander lucioperca)	4	4.60
Total		9	87	100.0

Table 3 show that the largest share of the ichthyopopulation in Boračko Lake consist of chub, the only representative of the Leuciscidae family, with a share of 43.7%. Fish from the Cyprinidae family make up 27.6% of the lake's ichthyopopulation, and representatives of this family (common carp and Prussian carp) are equally represented with 13.8% each. Fish from the Salmonidae family account for 12.6% of the total share – lake trout is represented with 6.9%, Ohrid trout with 4.6%, while peled makes up 1.1% of the total share. Gudgeon is the only representative of the Gobionidae family and its share is 4.6%. The Percidae family is represented by pike-perch, also with 4.6%, while a representative of the Cobitidae family (Neretva spined loach) participates with a share of 6.9% of the total ichthyopopulation of Boračko Lake. A total of 87 fish specimens were caught (Poljoprivredno-prehrambeni fakultet Sarajevo, 2018). When comparing ichthyological research conducted in 2018 with previous studies from 2003 and 2011, it can be concluded that no new fish species have been introduced.

In the latest ichthyological research of Boračko Lake conducted in 2023, a total of 11 fish species were registered: two from the Salmonidae family (lake trout and Ohrid trout); two from the Cyprinidae family (common carp and Prussian carp); three from the Leuciscidae family: chub, Eurasian minnow and bleak (*Alburnus alburnus*); and one species each from the Tincidae family (tench – *Tinca tinca*), from the Gobionidae family (gudgeon), from the Percidae family (pike-perch) and one species from the Cobitidae family (Neretva spined loach). Table 4. shows the qualitative and quantitative composition of the fish populations of Boračko Lake.

Table 4. Qualitative-quantitative composition of fish populations in Boračko L. (2023)

Order	Family	Species	Abundance	
			Absolute (n)	Relative (%)
Salmoniformes	Salmonidae	Lake trout (Salmo trutta)	10	5.70
		Ohrid trout (Salmo letnica)	2	1.20
Cypriniformes	Cyprinidae	Common carp (Cyprinus carpio)	29	16.70
		Prussian carp (Carassius gibelio)	31	17.80
	Leuciscidae	Chub (Squalius cephalus)	35	20.10
		Eurasian minnow (Phoxinus phoxinus)	16	9.20
		Bleak (Alburnus alburnus)	2	1.20
	Tincidae	Tench (Tinca tinca)	14	8.00
	Gobionidae	Gudgeon (Gobio gobio)	4	2.30
	Cobitidae	Neretva spined loach (Cobitis narentana)	6	3.40
Perciformes	Percidae	Pike-perch (Sander lucioperca)	25	14.40
Total		11	174	100.0

The latest ichthyological research conducted in 2023 shows that the largest share of the ichthyopopulation (over 1/3) in Boračko Lake consist of fish from the Cyprinidae family, with a total share of 34.5%. Within this family, Prussian carp is the most represented species (17.8%), followed by common carp with 16.7%. Fish from the Leuciscidae family make up a total of 30.5% of the ichthyopopulation, with chub being the most abundant species at 20.1%, followed by the Eurasian minnow with 9.2%, while bleak makes up a 1.2% share. Based on the latest research, considering individual fish species, chub is the most abundant species in Boračko Lake. Salmonid fish species are represented with a total share of only 6.9% – lake trout makes up 5.7%, while Ohrid trout makes up 1.2% of the share. Pike-perch, as the only representative of the Percidae family, participates with a share of 14.4%. The Tincidae family is represented by tench with 8%, the Gobionidae family is represented by gudgeon with a share of 2.3%, while the Cobitidae family is represented by Neretva spined loach and this species participates with a share of 3.4% of the total fish population in Boračko Lake. A total of 174 fish specimens were caught.
Compared to the results of previous studies conducted in 2003, 2011 and 2018 (Sofradžija *et al.,* 2003; Škrijelj *et al.,* 2011; Poljoprivredno-prehrambeni fakultet Sarajevo, 2018), 2 new fish species have been introduced into Boračko Lake (tench and bleak). It can also be concluded from recent research that there has been a significant increase in the population of the invasive and highly aggressive pike-perch, which, like

all other introduced fish species, is greatly affecting the constant decline of salmonid fish populations in Boračko Lake.

CONCLUSIONS

According to previous literary references (Mihić, 1985; Vuković, 1977; Vuković and Ivanović, 1971), Boračko Lake was inhabited exclusively by indigenous lake trout and the introduced Ohrid trout, which successfully adapted to the living conditions in the lake and even spawned there. However, at the end of the 20th and beginning of the 21st century, there was a massive, unprofessional and unplanned introduction of foreign fish species into Boračko Lake. Non-native species, in larger or smaller quantities, were introduced by irresponsible fishermen, with the aim of "improving" the lake's ichthyofauna. According to available relevant studies conducted in 2003, 2011, 2018 and 2023, at least 12 fish species have been introduced into Boračko Lake in the last 20 years, most of which are not naturally distributed in the Neretva River basin, i.e. the Adriatic Sea basin (except for the Eurasian minnow and Neretva spined loach). As highly adaptable fish species, and often also large predators such as pike-perch and chub, individuals of these species have become a significant danger for the survival of the native salmonid fish species in Boračko Lake. In addition, some cyprinid fish species, such as common carp and prussian carp, are hosts of numerous parasites and carriers of various diseases, meaning that there is a high possibility of mass illness and death of fish, especially indigenous species. Foreign species have been introduced into Boračko Lake that, due to their eco-biological and other characteristics, represent competitors and predators to indigenous fish, directly competing for the same living conditions, and have significantly disrupted the natural food chains and biological productivity pyramid of the lake. Therefore, it is necessary to implement planned remedial fishing (which is enabled by the Law on Freshwater Fisheries of the Federation of Bosnia and Herzegovina, Article 30.) and permanently remove the entire non-native population from Boračko Lake, creating conditions for the rehabilitation of the indigenous lake trout population.

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INTRODUKCIJA NOVIH VRSTA RIBA U BORAČKOM JEZERU U POSLJEDNJIH 20 GODINA

Sažetak

Boračko jezero je prirodno planinsko i ledničko jezero smješteno u podnožju planine Prenj na nadmorskoj visini od 397 m. Sa ihtiološkog aspekta, ovo je jezero izvorno bilo salmonidnog karaktera, naseljeno isključivo jezerskom pastrmkom. Međutim, u posljednjih nekoliko decenija ihtiofauna ovog jezera je značajno izmijenjena zbog introdukcije, odnosno uvođenja drugih vrsta riba. Ihtiološka istraživanja na Boračkom jezeru koja su obavljena 2003., 2011. i 2018. godine pokazala su na prisustvo nekoliko novih vrsta riba, među kojima preovladavaju ciprinidne vrste riba (ribe iz reda Cypriniformes). Registrovan je samo mali broj jedinki salmonidnih vrsta riba. Ihtiološkim istraživanjima, provedenim 2023. godine, potvrđeno je prisustvo nekoliko novih vrsta riba, koje nisu bile registrovane u ranijim istraživanjima. Danas u Boračkom jezeru živi 11 vrsta riba, među kojima su najbrojnije ciprinidne (7 vrsta) i one čine 75,3% ukupne ihtiopopulacije jezera, dok salmonide predstavljaju dvije vrste sa ukupnim udjelom od 6,9%. Ostatak ihtiopopulacije ovog jezera čini smuđ sa udjelom od 14,4%, dok neretvanski vijun učestvuje sa udjelom od 3,4% ukupne ihtiopopulacije. U narednom periodu je potrebno uvođenje sistemskog riješavanja problema, kroz selektivni izlov alohtonih i invazivnih vrsta riba kako bi se sačuvale, zaštitile i obnovile populacije autohtonih vrsta.

Ključne riječi: *Boračko jezero; salmonidi; ciprinidi; introdukcija; istraživanje*

FISH POPULATIONS IN FISHING WATERS IN THE AREA OF FOJNICA MUNICIPALITY*

Samir Muhamedagić[1], Dino Lepara*[1]

Original scientific paper

Abstract

The ichthyological research in this study aimed to determine the current state of fish populations and to obtain relevant data on the presence of certain fish species in the fishing waters of the Fojnica municipality. The field part of the ichthyological research in the fishing waters of the Fojnica municipality was conducted in October 2019, in the following watercourses: Fojnička River, Željeznica, Radava, Brložnjak, Dragača, Požarna, Borovnica, Hrastinčica, Kozica, Jezernica and Šćona. The fish were caught with a special-purpose electrofishing generator – model "Honda" OHV 5.5 (with a power of 3.0 kW). Collected fish samples were processed on-site, and all individuals were then returned to the watercourse from which they were caught. The ichthyological research in the fishing waters of the Fojnica municipality recorded 9 fish species from four families of freshwater ichthyofauna. Systematic determination revealed that the Salmonidae family is represented by 4 registered species (44.44%), while the Leuciscidae family is represented by 3 species (33.33%). The families Cyprinidae and Cottidae are represented with one registered species each (11.11%). Based on the analysis of the results from field research conducted at representative localities of the aforementioned watercourses, it can be concluded that the ecological conditions and the quantitative-qualitative composition of ichthyopopulations are at a satisfactory level for all indigenous fish species.

Keywords: *Fojnica; fishing waters; ichthyopopulation; electrofishing; ichthyological research*

INTRODUCTION

The richness and diversity of flora and fauna in Bosnia and Herzegovina are most clearly manifested through the abundance and diversity of fish species that inhabit its waters (Muhamedagić, 2019). Fish in Bosnia and Herzegovina are represented by 118 (sub)species, classified into 70 genera and 27 families. Of the 118 registered (sub)species, 105 are indigenous, and 13 (sub)species have been introduced into the waters of Bosnia and Herzegovina (Vuković, 1977; Sofradžija, 2009). The first ichthyological research in the waters of Bosnia and Herzegovina were conducted in the

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19th century, during the Austro-Hungarian Monarchy. The works of Heckel and Kner (1858), Steindachner (1882) and Heintz (1908) collected significant data on the distribution of salmonid fish species in this area and their biosystematic affiliation.
From the beginning of the 20th century until today, the ichthyofauna of Bosnia and Herzegovina has been a subject of interest for domestic and foreign ichthyologists and has been presented in numerous studies of the Yugoslav and European ichthyofauna (Ćurčić, 1915, 1916; Karaman, 1926, 1928, 1932, 1937, 1938, 1952; Taler, 1950, 1953a, 1953b; Vuković & Ivanović, 1971; Vuković, 1977; Vuković & Sofradžija, 1987; Kottelat, 1997; Kottelat & Freyhof, 2007; Sofradžija, 2009).
Ichthyological research in the Fojnička River basin has been conducted on several occasions over the last twenty years (Korjenić, 2003a, 2003b, 2004a, 2004b, 2005a, 2005b, 2005c, 2006, 2009; Korjenić & Sofradžija, 2005; Korjenić *et al.,* 2009; Žujo & Korjenić, 2005; Škrijelj *et al.,* 2008; Pavličević *et al.,* 2014). In ichthyological research conducted in 2008, a total of 6 fish species from 4 families of freshwater ichthyofauna were registered in the fishing waters of the Fojnica municipality (Škrijelj *et al.,* 2008).
The goal of the ichthyological research in this study is to determine the current status of fish populations and to obtain relevant data on the representation of certain fish species in the fishing waters of the Fojnica municipality. The results of the ichthyological analyses regarding the status of fish populations, their qualitative-quantitative structure, and their comparison with the status of these populations during previous studies in these fishing waters should provide an overview of the fish stock, specifically whether a population is declining, stagnating or increasing.

MATERIALS AND METHODS

The field part of the ichthyological research of fishing waters in the Fojnica municipality was conducted from October 29 to 30, 2019, and the fishing waters as well as the localities where the research was carried out are presented in Table 1.
Fish were caught using an electrofishing generator "Honda" OHV 5.5, with a power of 3.0 kW. The collected fish samples were processed in the field and returned to their natural habitat, while a smaller number of representative specimens were fixed in 4% formaldehyde and transported to the laboratory of the Center for Aquaculture and Fisheries at the Faculty of Agriculture and Food Sciences in Sarajevo for further analysis.

Table 1. Processed water surfaces

Fishing water	Locality	Length of the locality (m)	Width of the locality (m)
Fojnička River	Šćitovo	100.0	12.0
Željeznica	Bakovići	100.0	8.0
Radava	Praje	100.0	3.0
Brložnjak	Dusina	100.0	3.0
Dragača	Urban zone	100.0	6.0
Požarna	Požarna	100.0	5.0
Borovnica	Tovarište	100.0	3.0
Hrastinčica	Tovarište	100.0	2.0
Kozica	Mehurići	100.0	2.0
Jezernica	Mouth of the Razdolja	100.0	3.0
Šćona	Pavlovac	100.0	4.0

The systematic determination of fish was conducted according to Vuković & Ivanović (1971): Ribe Bosne i Hercegovine; and Kottelat & Freyhof (2007): Handbook of European Freshwater Fishes.

RESULTS AND DISCUSSION

The municipality of Fojnica is located approximately between 43°47' and 44°28' N, which practically means it is located almost halfway between the equator and the northern geographic pole. The main characteristic of the hydrographic network in the Fojnica municipality is its overall territorial affiliation with the Black Sea river basin. The backbone of this hydrographic network is the Fojnička River. The total length of all watercourses in the Fojnica municipality, determined by planimetry of the lengths of watercourses from topographic maps at a scale of 1:25 000, is approximately 170.00 km. Accordingly, the average length of the main watercourses is about 0.57 km/km², which is slightly above the Bosnian-Herzegovinian average.

Fojnička River

The quantitative-qualitative composition of the ichthyofauna of the Fojnička River (locality Šćitovo) and the proportion of individual fish species in the total ichthyopopulation are presented in Table 2 and Figure 1.

Table 2. Qualitative-quantitative composition of the ichthyopopulation of the Fojnička River in the locality of Šćitovo

Species	Quantity	Body weight (g)			Total length (cm)	
		max	min	Σ	max	min
Brown trout	60	862.0	226.0	32 640.0	45.0	30.0
Rainbow trout	1	30.0	30.0	30.0	15.0	15.0
Huchen	4	4 520.0	488.0	10 016.0	72.5	38.0
Grayling	34	158.0	9.0	2 839.0	27.0	8.0
Chub	37	288.0	7.0	5 457.5	28.0	8.5
Eurasian minnow	120	10.0	6.0	960.0	9.0	7.0
Schneider	162	18.0	11.0	2 349.0	12.5	8.5
Danube barbel	13	26.0	18.0	286.0	21.5	16.0
Bullhead	40	11.0	7.0	360.0	11.5	9.5
Total	471	-	-	54 937.5	-	-

At the Šćitovo locality, in the Fojnička River, nine fish species have been recorded: four from the Salmonidae family (brown trout – *Salmo trutta*, rainbow trout – *Oncorhynchus mykiss*, huchen – *Hucho hucho* and grayling – *Thymallus thymallus*), three from the Leuciscidae family (chub – *Squalius cephalus*, Eurasian minnow – *Phoxinus phoxinus* and schneider – *Alburnoides bipunctatus*), as well as one species each from the Cyprinidae family (Danube barbel – *Barbus balcanicus*) and the Cottidae family (bullhead – *Cottus gobio*). A total of 471 specimens were caught, with a total body weight of 54.9 kg.

Compared to ichthyological research conducted for the purpose of developing the fisheries baseline document (Škrijelj *et al.,* 2008) and the revision of the fisheries baseline document (Pavličević *et al.,* 2014), four new fish species were recorded in this watercourse: huchen, rainbow trout, chub and schneider

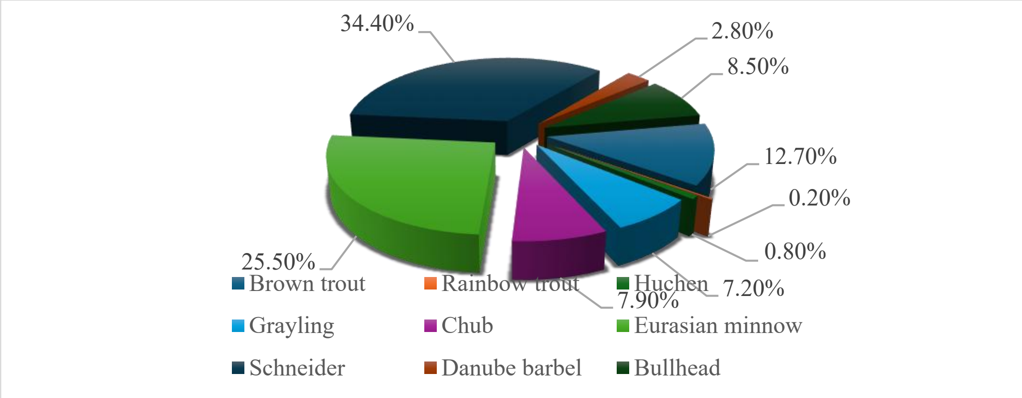

Figure 1. Qualitative-quantitative composition of the ichthyopopulation of the Fojnička River in the locality of Šćitovo

The largest share of the ichthyopopulation in the Fojnička River, at the Šćitovo locality, is made up of fish species from the Leuciscidae family (67.8%), of which the most common are schneider (34.4%) and Eurasian minnow (25.5%), while chub makes up 7.9% of the total ichthyopopulation. Salmonid fish species are represented in this locality with a share of 20.9% of the total ichthyopopulation. Brown trout is represented with a share of 12.7%, grayling with 7.2%, huchen with 0.8% and rainbow trout with 0.2% in the total ichthyopopulation. Bullhead, as the only representative of the Cottidae family, is represented with a share of 8.5%. A not so significant share of the ichthyopopulation is made up of the Danube barbel, a representative of the Cyprinidae family, with only 2.8% of the total ichthyopopulation of the Fojnička River at the Šćitovo locality.

Željeznica River

The quantitative-qualitative composition of the ichthyofauna of the Željeznica River (locality Bakovići) and the proportion of individual fish species in the total ichthyopopulation are presented in Table 3 and Figure 2.

Table 3. Qualitative-quantitative composition of the ichthyopopulation of the Željeznica River in the locality Bakovići

Species	Quantity	Body weight (g)			Total length (cm)	
		max	min	Σ	max	min
Brown trout	107	372.0	18.0	20 865.0	37.5	13.5
Grayling	46	286.0	18.0	6 992.0	33.5	14.0
Bullhead	26	12.0	8.0	260.0	11.5	9.0
Total	179	-	-	28 117.0	-	-

In the Željeznica River, at the Bakovići locality, three fish species have been registered: brown trout (*Salmo trutta*) and grayling (*Thymallus thymallus*) from the Salmonidae family, and bullhead (*Cottus gobio*) from the Cottidae family. A total of 179 specimens were caught, with a total body weight of 28.1 kg.
During this research, populations of rainbow trout, Danube barbel and Eurasian minnow were not recorded, but they were present in the overall ichthyopopulation in earlier studies (Škrijelj *et al.,* 2008; Pavličević *et al.,* 2014).

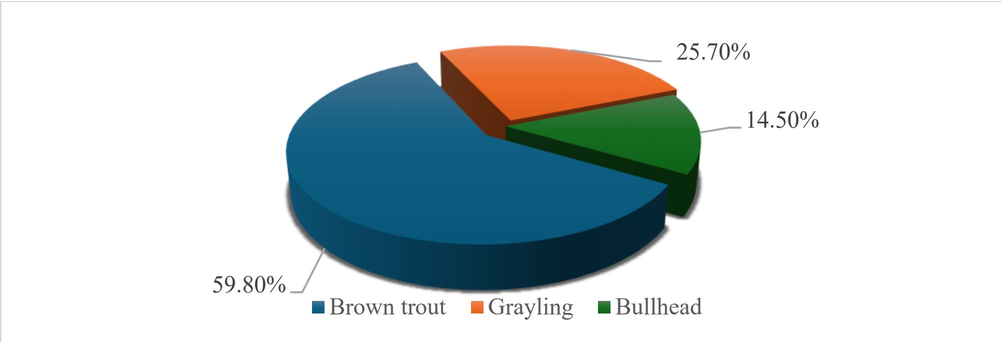

Figure 2. Qualitative-quantitative composition of the ichthyopopulation of the Željeznica River in the locality Bakovići

The largest share of the total ichthyopopulation in the Željeznica River, at the Bakovići locality, is made up of salmonid fish species – brown trout and grayling. Brown trout is represented with a share of 59.8%, and grayling with a share of 25.7% in the total ichthyopopulation, meaning that salmonids comprise 85.5% of the total ichthyopopulation at this locality. Bullhead is represented with a share of 14.5%.

Radava River
The quantitative-qualitative composition of the ichthyofauna of the Radava River (locality Praje) and the proportion of individual fish species in the total ichthyopopulation are presented in Table 4 and Figure 3.

Table 4. Qualitative-quantitative composition of the ichthyopopulation of the Radava River in the locality of Praje

Species	Quantity	Body weight (g)			Total length (cm)	
		max	min	Σ	max	min
Brown trout	52	22.0	6.0	728.0	13.5	8.5
Rainbow trout	2	12.0	9.0	21.0	9.5	7.5
Bullhead	24	22.0	9.0	372.0	12.0	10.0
Total	78	-	-	1121.0	-	-

In the Radava River, three fish species have been recorded: two from the Salmonidae family (brown trout – *Salmo trutta* and rainbow trout – *Oncorhynchus mykiss*) and one species from the Cottidae family (bullhead – *Cottus gobio*). A total of 78 fish specimens were caught, with a total body weight of 1.1 kg.

In addition to brown trout and bullhead, species recorded during the studies conducted in 2008 (Škrijelj *et al.,* 2008) and 2014 (Pavličević *et al.,* 2014), this research also recorded two specimens of rainbow trout, which likely escaped from a fish farm.

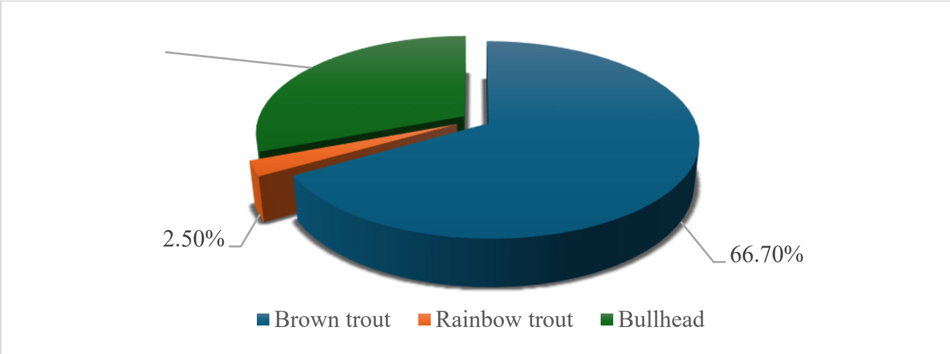

Figure 3. Qualitative-quantitative composition of the ichthyopopulation of the Radava River in the locality of Praje

The largest share of the ichthyopopulation in the Radava River, at the Praje locality, is made up of salmonid fish species, with a share of 69.2%. The most represented is the brown trout, with a share of 66.7%, while the rainbow trout makes up 2.5% of the total ichthyopopulation. A significant part of the ichthyopopulation in this area is also composed of the bullhead, with a 30.8% share of the total ichthyopopulation in the Radava River at the Praje locality.

Brložnjak Stream

The quantitative-qualitative composition of the ichthyofauna of the Brložnjak Stream (locality Dusina) and the proportion of individual fish species in the total ichthyopopulation are presented in Table 5 and Figure 4.

Table 5. Qualitative-quantitative composition of the ichthyopopulation of the Brložnjak Stream in the locality of Dusina

Species	Quantity	Body weight (g)			Total length (cm)	
		max	min	Σ	max	min
Brown trout	36	31.0	10.0	738.0	15.0	9.5
Bullhead	16	19.0	9.0	224.0	11.0	9.5
Total	52	-	-	962.0	-	-

In the Brložnjak Stream, two fish species have been recorded: one from the Salmonidae family (brown trout – *Salmo trutta*) and one from the Cottidae family (bullhead – *Cottus gobio*). A total of 52 fish specimens were caught, with a total body weight of approximately 1.0 kg.

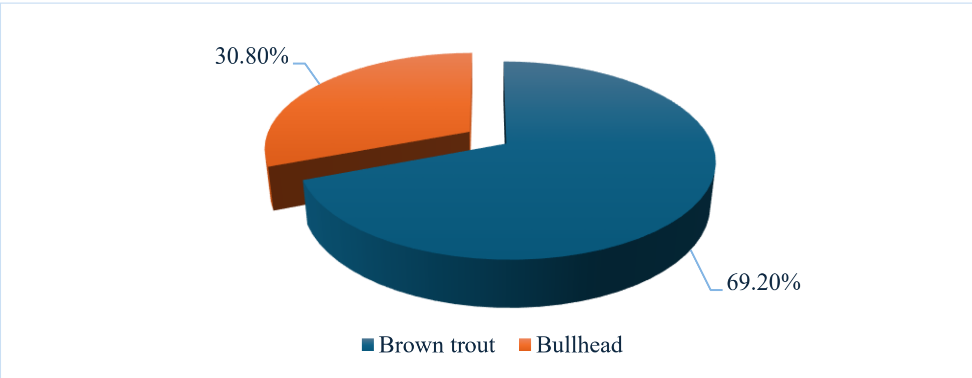

Figure 4. Qualitative-quantitative composition of the ichthyopopulation of the Brložnjak Stream in the locality of Dusina

The largest share, about 2/3 of the total ichthyopopulation in the Brložnjak Stream, at the Dusina locality, is made up of brown trout (69.2%), of all age categories. The rest of the ichthyopopulation is made up of bullhead, with a 30.8% share in the total ichthyopopulation of the Brložnjak Stream, at the Dusina locality.

Dragača River

The quantitative-qualitative composition of the ichthyofauna of the Dragača River, in the urban zone area, and the proportion of individual fish species in the total ichthyopopulation are presented in Table 6 and Figure 5.

Table 6. Qualitative-quantitative composition of the ichthyopopulation of the Dragača River, urban zone

Species	Quantity	Body weight (g)			Total length (cm)	
		max	min	Σ	max	min
Brown trout	95	840.0	32.0	41 420.0	41.0	15.0
Rainbow trout	2	905.0	49.0	954.0	38.5	17.0
Bullhead	28	22.0	5.0	378.0	12.5	7.0
Total	125	-	-	42752.0	-	-

In the Dragača River, three fish species have been recorded: two species from the Salmonidae family (brown trout – *Salmo trutta* and rainbow trout – *Oncorhynchus mykiss*) and one from the Cottidae family (bullhead – *Cottus gobio*). A total of 125 fish specimens were caught, with a total body weight of 42.7 kg.

Compared to the research from 2008 (Škrijelj *et al.,* 2008), it can be concluded that there were no significant changes in the composition of the ichthyopopulation of the Dragača River.

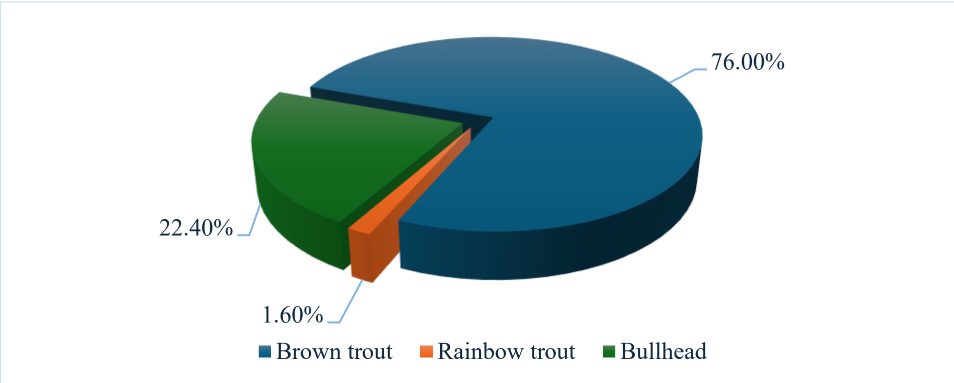

Figure 5. Qualitative-quantitative composition of the ichthyopopulation of the Dragača River (urban zone)

The largest share of the ichthyopopulation in the Dragača River (the urban zone area) is made up of brown trout with a share of 76.0% of the total ichthyopopulation. Rainbow trout is represented with a share of 1.6%. Therefore, salmonids are represented with a share of 77.6% in the total ichthyopopulation. Bullhead is represented with a share of 22.4% in the total ichthyopopulation of the Dragača River, in the urban zone area.

Požarna River
The quantitative-qualitative composition of the ichthyofauna of the Požarna River (locality Požarna) and the proportion of individual fish species in the total ichthyopopulation are presented in Table 7 and Figure 6.

Table 7. Qualitative-quantitative composition of the ichthyopopulation of the Požarna River in the locality of Požarna

Species	Quantity	Body weight (g)			Total length (cm)	
		max	min	Σ	max	min
Brown trout	93	105.0	10.0	5 347.5	21.0	8.0
Bullhead	33	34.0	11.0	742.5	12.0	7.0
Total	126	-	-	6 090.0	-	-

In the Požarna River, at the Požarna locality, two species of fish were registered: one from the Salmonidae family (brown trout – *Salmo trutta*) and one species from the Cottidae family (bullhead – *Cottus gobio*). A total of 126 fish specimens were caught, with a total body weight of about 6.0 kg.

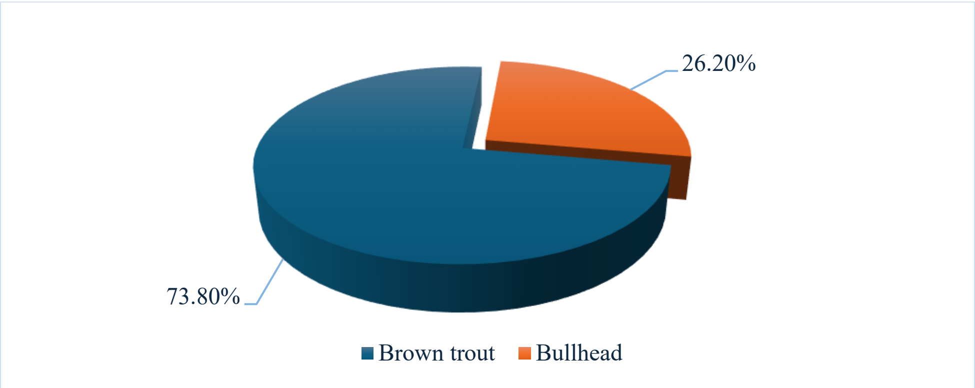

Figure 6. Qualitative-quantitative composition of the ichthyopopulation of the Požarna River in the locality of Požarna

The largest share of the total ichthyofauna in the Požarna River is made up of brown trout, accounting for 73.8%. The remaining ichthyofauna is composed of the bullhead, with a share of 26.2% in the total ichthyofauna of the Požarna River, at the Požarna locality.

Borovnica River

The quantitative-qualitative composition of the ichthyofauna of the Borovnica River (locality Tovarište) and the proportion of individual fish species in the total ichthyopopulation are presented in Table 8 and Figure 7.

Table 8. Qualitative-quantitative composition of the ichthyopopulation of the Borovnica River (locality Tovarište)

Species	Quantity	Body weight (g)			Total length (cm)	
		max	min	Σ	max	min
Brown trout	93	24.0	6.0	1 395.0	13.5	7.5
Bullhead	12	18.0	7.0	150.0	10.5	7.5
Total	105	-	-	1 545.0	-	-

In the Borovnica River, two species of fish have been recorded: one from the Salmonidae family (brown trout – *Salmo trutta*) and one from the Cottidae family (bullhead – *Cottus gobio*). A total of 105 fish specimens were caught, with a total body weight of approximately 1.5 kg.

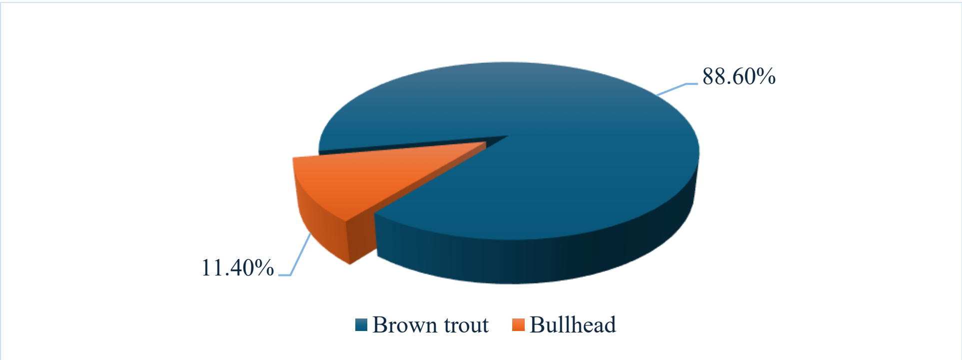

Figure 7. Qualitative-quantitative composition of the ichthyopopulation of the Borovnica River in the locality of Tovarište

The largest share in the Borovnica River (Tovarište locality) is made up of brown trout (88.6%), of all age categories. The rest of the ichthyofauna is made up of bullhead, with a share of 11.4% in the total fish population of the Borovnica River.

Hrastinčica River
The quantitative-qualitative composition of the ichthyofauna of the Hrastinčica River (locality Tovarište) and the proportion of individual fish species in the total ichthyopopulation are presented in Table 9.

Table 9. Qualitative-quantitative composition of the ichthyopopulation of the Hrastinčica River (locality Tovarište)

Species	Quantity	Body weight (g)			Total length (cm)	
		max	min	Σ	max	min
Brown trout	48	15.0	4.0	456.0	11.0	7.0
Total	48	-	-	456.0	-	-

Only one fish species belonging to the Salmonidae family (brown trout – *Salmo trutta*) has been registered in the Hrastinčica River. A total of 48 specimens of this species were caught, with a total body mass of about 0.5 kg.

Kozica River
The quantitative-qualitative composition of the ichthyofauna of the Kozica River (locality Mehurići) and the proportion of individual fish species in the total ichthyopopulation are presented in Table 10.

Table 10. Qualitative-quantitative composition of the ichthyopopulation of the Kozica River in the locality of Mehurići

Species	Quantity	Body weight (g)			Total length (cm)	
		max	min	Σ	max	min
Brown trout	30	14.0	9.0	345.0	11.0	8.5
Total	30	-	-	345.0	-	-

In the Kozica River, one fish species has been recorded, the brown trout (*Salmo trutta*), which belongs to the Salmonidae family. A total of 30 specimens of this fish were caught, with a total body weight of approximately 0.3 kg.

Jezernica River
The quantitative-qualitative composition of the ichthyofauna of the Jezernica River (locality mouth of the Razdolja) and the proportion of individual fish species in the total ichthyopopulation are presented in Table 11 and Figure 8.

Table 11. Qualitative-quantitative composition of the ichthyopopulation of the Jezernica River (mouth of the Razdolja)

Species	Quantity	Body weight (g)			Total length (cm)	
		max	min	Σ	max	min
Brown trout	87	42.0	5.0	2 044.5	16.5	7.5
Bullhead	30	11.0	3.0	210.0	10.0	6.0
Total	117	-	-	2 254.5	-	-

In the Jezernica River (mouth of the Razdolja locality), two fish species have been recorded: one from the Salmonidae family (brown trout – *Salmo trutta*) and one from the Cottidae family (bullhead – *Cottus gobio*). A total of 117 fish specimens were caught, with a total body weight of approximately 2.2 kg.

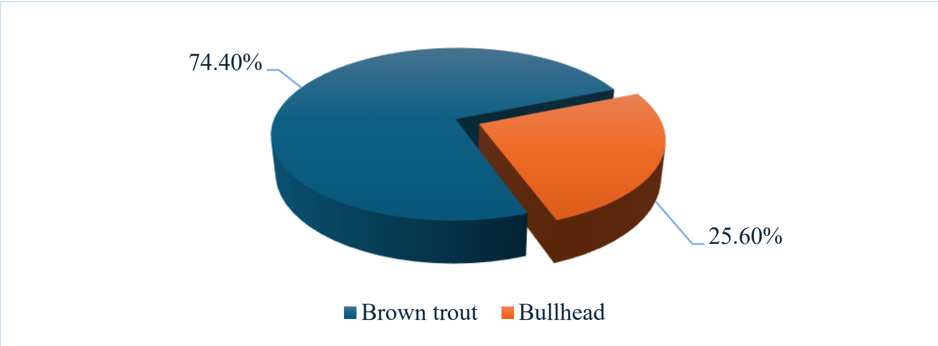

Figure 8. Qualitative-quantitative composition of the ichthyopopulation of the Jezernica River in the locality mouth of the Razdolja

The largest share of the total ichthyofauna in the Jezernica River, at the mouth of Razdolja locality, is made up of brown trout, accounting for 74.4%. The remaining ichthyopopulation is composed of the bullhead, with a share of 25.6% in the total ichthyofauna of the Jezernica River.

Šćona River

The quantitative-qualitative composition of the ichthyofauna of the Šćona River (locality Pavlovac) and the proportion of individual fish species in the total ichthyopopulation are presented in Table 12 and Figure 9.

Table 12. Qualitative-quantitative composition of the ichthyopopulation of the Šćona River in the locality of Pavlovac

Species	Quantity	Body weight (g)			Total length (cm)	
		max	min	Σ	max	min
Brown trout	59	74.0	10.0	2 478.0	18.5	10.5
Bullhead	34	23.0	5.0	476.0	12.0	6.5
Total	93	-	-	2 954.0	-	-

Two fish species were registered in the Šćona River: one from the Salmonidae family (brown trout - *Salmo trutta*) and one from the Cottidae family (bullhead – *Cottus gobio*). A total of 93 fish were caught, with a total body weight of about 3.0 kg.

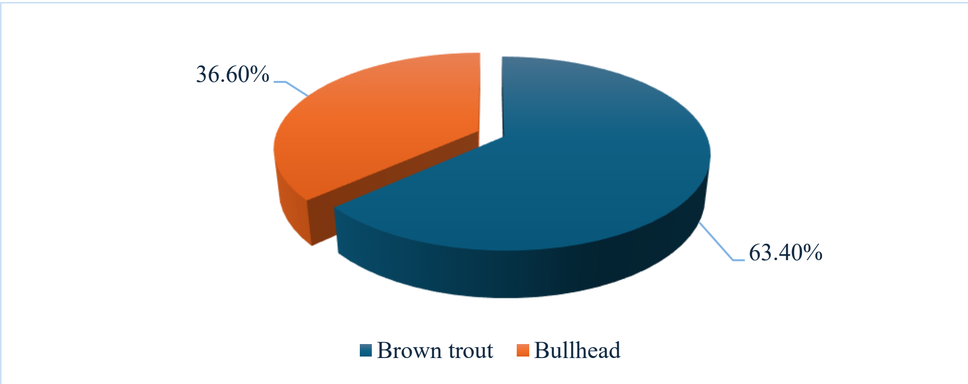

Figure 9. Qualitative-quantitative composition of the ichthyopopulation of the Šćona River in the locality of Pavlovac

The largest share of the total fish population in the Šćona River is made up of brown trout (63.4%), of all age categories. The rest of the fish population is made up of bullhead, with a 36.6% share in the total fish population of the Šćona River (Pavlovac locality).

Summary analysis of the qualitative-quantitative structure of the ichthyopopulation

In ichthyological research of fishing waters in the area of Fojnica municipality, 9 fish species from four families of freshwater ichthyofauna were registered. The results of the qualitative composition of the ichthyofauna of fishing waters in the area of Fojnica municipality are presented in table 13 and figures 10 and 11.

Table 13. Systematic review of the ichthyopopulation of fishing waters in the area of Fojnica municipality

Family	Species
Salmonidae	Brown trout (*Salmo trutta*)
	Huchen (*Hucho hucho*)
	Grayling (*Thymallus thymallus*)
	Rainbow trout (*Oncorhynchus mykiss*)
Leuciscidae	Chub (*Squalius cephalus*)
	Eurasian minnow (*Phoxinus phoxinus*)
	Schneider (*Alburnoides bipunctatus*)
Cyprinidae	Danube barbel (*Barbus balcanicus*)
Cottidae	Bullhead (*Cottus gobio*)

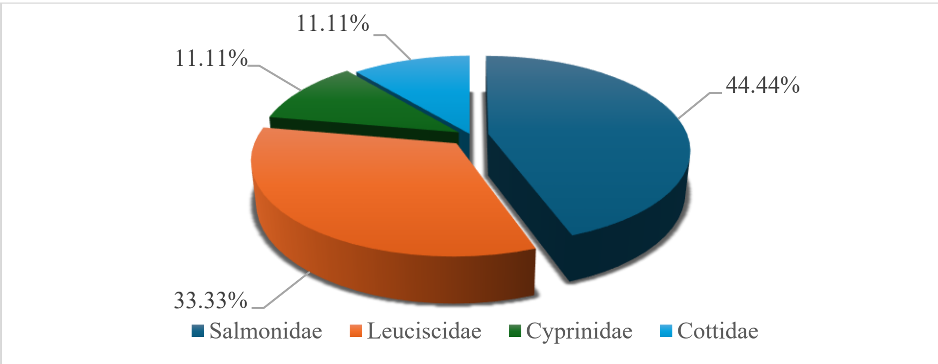

Figure 10. Percentage representation of the ichthyofauna of fishing waters in the area of Fojnica municipality

The salmonid family is represented by four species (44.44%) in the fishing waters of Fojnica municipality – three indigenous species and one allochthonous (non-native) species. Brown trout (*Salmo trutta*), huchen (*Hucho hucho*) and grayling (*Thymallus thymallus*) are indigenous species, while rainbow trout (*Oncorhynchus mykiss*) is allochthonous fish species. The Leuciscidae family is represented in these fishing waters by 3 registered species – 33.33% (chub – *Squalius cephalus*, Eurasian minnow – *Phoxinus phoxinus* and schneider – *Alburnoides bipunctatus*). With one species each (11.11%), the family Cyprinidae (Danube barbel – *Barbus balcanicus*) and the family Cottidae (bullhead – *Cottus gobio*) are represented in the fishing waters of Fojnica municipality.
During field research conducted in 2008 (Škrijelj *et al.,* 2008) and 2014 (Pavličević *et al.,* 2014) in the Fojnica municipality area, a total of six fish species from four families

of freshwater ichthyofauna were recorded: Salmonidae, Leuciscidae, Cyprinidae and Cottidae. The Salmonidae family was represented by three species (50.0%) – brown trout, grayling and rainbow trout. Other families were represented by one species each (16.67%) – the Leuciscidae family was represented by the Eurasian minnow, the Cyprinidae family by the Danube barbel, while the Cottidae family was represented by the bullhead. Comparing all three studies, it can be concluded that the latest research recorded three new fish species (huchen, chub and schneider) that were not registered in the previous studies from 2008 and 2014.

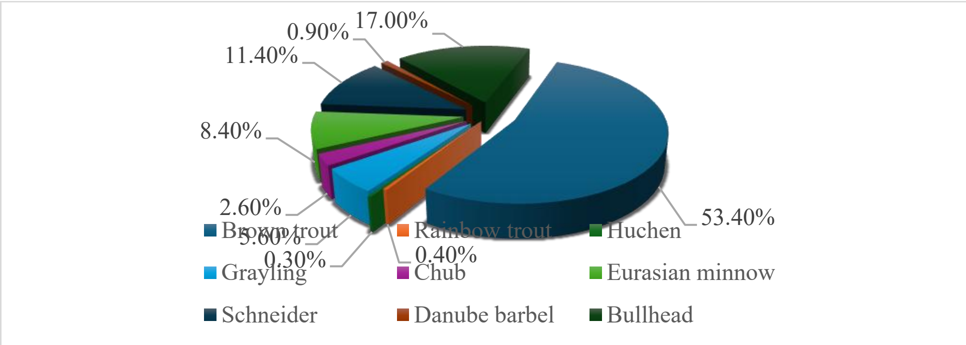

Figure 11. Percentage representation of the ichthyopopulations of investigated watercourses in the area of Fojnica municipality

The majority of the total ichthyopopulation of the studied watercourses in the Fojnica municipality area is made up of fish from the Salmonidae family with a total share of 59.7%. Therefore, fish from this family account for more than half of the total ichthyopopulation in this area – brown trout is the most dominant species representing 53.4%, followed by grayling with a share of 5.6%, rainbow trout with 0.4% and huchen with only 0.3%. Fish from the Leuciscidae family are represented with a total share of 22.4% – schneider with a share of 11.4%, European minnow with 8.4% and chub with a share of 2.6%. The rest of the ichthyopopulation of the studied watercourses in the Fojnica municipality includes the Danube barbel (Cyprinidae family) with a share of 0.9% and the bullhead (Cottidae family) with a share of 17,0%.

CONCLUSIONS

Based on the analysis of the results of field research conducted at representative localities on the rivers: Željeznica, Radava, Dragača, Požarna, Borovnica, Hrastinčica, Kozica, Jezernica, Šćona and the Fojnička River, as well as on the Brložnjak Stream, it can be concluded that the ecological conditions and the quantitative-qualitative composition of the ichthyopopulations are at a satisfactory level for all indigenous fish species. In the ichthyological research of fishing waters in the area of Fojnica municipality, 9 fish species from four families of freshwater ichthyofauna were recorded: Salmonidae, Leuciscidae, Cyprinidae and Cottidae. Therefore, the results of the ichthyological research have undoubtedly shown that the current state of the fish stock in these waters is at a satisfactory level, especially regarding the populations of brown trout, huchen and grayling. Compared to the results of the ichthyological research conducted in 2008 for the purpose of developing the fisheries baseline document (Škrijelj *et al.,* 2008) and research conducted in 2014 for the purpose of developing the revision of the fisheries baseline document (Pavličević *et al.,* 2014), there have been no significant changes in the quantitative and/or qualitative composition of ichthyopopulations at most of the localities where research for this study was conducted. Significant changes in qualitative composition only occurred in the Fojnička River, where 4 new species were registered. In some watercourses and at certain localities, there has been an increase in the abundance of certain populations, leading to the conclusion that systematic efforts have been made in the past period to implement measures for the protection and improvement of fish stocks in the fishing waters of Fojnica municipality. It is very important to continue the current activities in the implementation of planned development measures, conservation and improvement of fish stocks, i.e. the diversity of ichthyopopulations in general, and one such measure is the stocking of fishing waters.

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POPULACIJE RIBA U RIBOLOVNIM VODAMA NA PODRUČJU OPĆINE FOJNICA

Sažetak

Ihtiološka istraživanja u ovoj studiji imala su za cilj utvrđivanje trenutnog stanja populacija riba, odnosno dobijanje relevantnih podataka o zastupljenosti pojedinih vrsta riba u ribolovnim vodama na području općine Fojnica. Terenski dio ihtioloških istraživanja ribolovnih voda na području općine Fojnica obavljen je tokom oktobra 2019. godine, na sljedećim vodotocima: Fojnička rijeka, Željeznica, Radava, Brložnjak, Dragača, Požarna, Borovnica, Hrastinčica, Kozica, Jezernica i Šćona. Izlov ribe je obavljen elektroagregatom za posebne namjene elektroribolova marke „Honda" OHV 5,5 (jačine 3.0 kW). Na terenu su obrađeni prikupljeni uzorci ribe, a zatim su sve jedinke vraćene u vodotok odakle su i izlovljene. U ihtiološkim istraživanjima ribolovnih voda na području općine Fojnica registrovano je 9 vrsta riba iz četiri porodice slatkovodne ihtiofaune. Sistematskom determinacijom je utvrđeno da je porodica Salmonidae zastupljena sa 4 registrovane vrste (44,44%), dok je porodica Leuciscidae zastupljena sa 3 vrste (33,33%). Sa po jednom registrovanom vrstom (11,11%) zastupljene su porodice Cyprinidae i Cottidae. Na osnovu analize rezultata terenskih istraživanja, provedenih na reprezentativnim lokalitetima gore navedenih vodotoka, može se konstatovati da su ekološki uslovi i kvantitativno-kvalitativni sastav ihtiopopulacija na zadovoljavajućem nivou za sve autohtone vrste riba.

Ključne riječi: *Fojnica; ribolovne vode; ihtiopopulacija; elektroribolov; ihtiološka istraživanja*

CONTRIBUTION TO THE KNOWLEDGE OF THE DIVERSITY OF CYANOBACTERIA AND ALGAE IN SELECTED PROTECTED AREAS OF THE CANTON OF SARAJEVO

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Original scientific paper

Abstract

The Canton Sarajevo has the highest number of protected natural areas, among the other cantons in the Federation of Bosnia and Herzegovina, including natural monument and protected landscape as follows: Skakavac, Bijambare, Vrelo Bosne, Trebević and Bentbaša. Each of these areas is characterized by specific geomorphological, hydrological, pedological, biological and ecological features, serving as centers of biological diversity for flora, fauna and fungi. This paper provides insights into the diversity of cyanobacteria and algae within selected protected areas of the Canton Sarajevo. To date, 268 taxa of cyanobacteria and algae have been identified within these areas. Bijambare boasts the highest species count (155), followed by Vrelo Bosne (66) and Skakavac (66). Data on the diversity of these organisms in Bentbaša and Trebević are currently unavailable, highlighting the need for more detailed and systematic research in these areas. Beyond establishing a list of cyanobacteria and algae, continuous monitoring of their habitats in the protected areas of Canton Sarajevo is essential. Given that cyanobacteria and algae are excellent bioindicators of aquatic ecosystems, incorporating them into planning documents for ongoing monitoring is a crucial step in preserving these valuable regions.

Key words: *algae, biodiversity, cyanobacteria, monitoring, protected areas*

INTRODUCTION

According to the IUCN definition, a protected area is a clearly defined geographical space recognized, dedicated, and managed through legal or other effective means to achieve the long-term conservation of nature, along with associated ecosystem services and cultural values. Although Bosnia and Herzegovina adheres to international categorization of protected areas, these categories are not fully harmonized within environmental protection laws across its administrative entities. Management of protected areas in Bosnia and Herzegovina is aligned with the institutional distribution

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of responsibilities for environmental and nature conservation (Barudanović *et al.,* 2024; Đurić *et al*., 2024).

The total area of protected lands in Bosnia and Herzegovina is notably low compared to other countries in the region and globally. The global target in the past decade was to protect 17% of terrestrial and 10% of aquatic territory in each country. According to official sources such as the *Federal Ministry of Envrionment and Tourism of Bosnia and Herzegovina* (http://e-prirodafbih.ba) and the *Republic Institute for the Protection of Cultural, Historical, and Natural Heritage* (http://e-priroda.rs.ba), the current official data indicate that approximately 3,13% of the national territory is under protection.

It is important to point out that the goal is to protect at least 30% of land and sea areas. This goal was formalized in December 2022 with the adoption of the Kunming-Montreal Global Biodiversity Framework (Kunming-Montreal Global Biodiversity Framework) during the Conference of Parties (COP15) of the United Nations Convention on Biological Diversity. Goal 3 of this framework calls on countries to "ensure and enable at least 30% of terrestrial, freshwater, coastal and marine areas, especially those of special importance for biological diversity and ecosystem functions and services, to be effectively preserved and managed" by 2030 (CBD, 2022).

In the Republika Srpska entity, there are 33 protected areas: two strict nature reserves (592.82 ha), three national parks (26,275.20 ha), 16 natural monuments (1,552.65 ha), three protected habitats (1,159.76 ha), five nature parks (38,340.46 ha), and three protected areas with sustainable use of natural resources (66.07 ha), totaling 73,023.33 ha or 2.96% of the entity’s territory (Đurić *et al*., 2024).

In the Federation of Bosnia and Herzegovina, the protected areas include one national park (36,629.08 ha), four natural monuments (9,207 ha), two nature parks (43,624.07 ha), and five protected landscapes (14,415.59 ha), totaling 103,875.74 ha across 12 designated areas. The most recently designated area is the Vjetrenica, Popovo Polje Protected Landscape, covering 4,712.19 ha. Overall, protected areas account for 3.98% of the Federation’s territory (Đurić *et al*., 2024).

It is important to note that the Brčko District has yet to designate any protected areas (Đurić *et al*., 2024).

Regarding special levels of protection in Republika Srpska, three areas have been recognized under international agreements and conventions: one Ramsar site (Bardača Wetland Complex), one UNESCO World Heritage natural site (the “Perućica” Primeval Forest), and the “Janj” Forest, which is part of the UNESCO heritage listing “Ancient and Primeval Beech Forests of the Carpathians and Other Regions of Europe.” In contrast, the Federation of Bosnia and Herzegovina has slightly fewer internationally designated areas, with Hutovo Blato and Livanjsko Polje listed as internationally important wetlands under the Ramsar Convention (Đurić *et al*., 2024).

The Vjetrenica Cave was officially registered on the UNESCO World Heritage List in 2024, during the 46th session of the World Heritage Committee. This decision was made on the basis of criteria related to exceptional universal value in terms of the preservation of natural diversity. Vjetrenica is recognized as one of the world's most important habitats for underground fauna, with numerous endemic and endangered species. It is

also an exceptionally well-preserved example of Dinaric karst and biodiversity (UNSECO, 2024).
The aim of this study is to establish a database and provide a review of the diversity of cyanobacteria and algae within protected areas, in order to support future inventory efforts and to integrate these findings into the monitoring of aquatic ecosystems.

MATERIALS AND METHODS

Study Area

Sarajevo Canton is one of the ten cantons within the Federation of Bosnia and Herzegovina, covering an area of 1,277 km². The canton stretches approximately 63.5 km along its longest axis, with an average width of about 20.5 km and a maximum width of roughly 55.6 km in its southern part (Drešković *et al*., 2015).
From a geotectonic perspective, most of Sarajevo Canton lies within the central Dinarides region, characterized by Paleozoic clastics, Mesozoic carbonates, Jurassic-Cretaceous flysch, and Neogene molasse. To the north, it borders the inner Dinarides, which are composed of Paleozoic and Neogene clastics and carbonates, a diabase-chert formation with ophiolites and mélange, Tertiary flysch, granite, andesite, dacite, tuff, and Tertiary molasse. The central, western, and northwestern portions of the canton belong morphostructurally to the Sarajevo-Zenica Basin. This basin is shaped by the Sarajevo-Busovača fault, oriented in a northwest-southeast direction. The basin and the surrounding mountains, Igman, Bjelašnica, Treskavica, Jahorina (to the south and southeast), Ozren and Bukovik (to the northeast), and Čemerska Mountain and Zvijezda (to the northwest), were formed during the Alpine orogenic phase and are part of the younger folded mountain systems of the Eurasian orogeny.

Geologically, the central part of the canton is covered by Quaternary deposits and Upper Miocene and Middle-to-Upper Miocene Cenozoic sediments including sandstones, marls, clays, limestones, and conglomerates. Lower and Middle Triassic deposits (Anisian and Ladinian) are found in the eastern and northern parts of the canton. The far northeastern section contains dolomites and limestones interbedded with chert, tuff, siliceous sandstones, marls, and calcarenites from the Middle and Lower Triassic.
The Bjelašnica and Igman Mountains also contain Middle and Lower Triassic deposits, transitioning southward (toward Treskavica) into Upper Triassic formations (limestones and dolomites with marl and sandstone layers), and eventually into Jurassic-Cretaceous flysch in the far southeast. The southwestern part of the canton features a highly complex geological structure that includes Silurian-Devonian, Devonian-Carboniferous, Permo-Triassic, and various Triassic deposits (Ćičić, 2002).
Climatically, Sarajevo Canton belongs to the southern portion of the northern temperate zone. The lower elevations are classified as Cfb (temperate oceanic climate with warm summers), while higher elevations transition to Dfb (humid continental climate with warm summers), and eventually to Dfc (subarctic climate with cool summers) on the highest mountain peaks.

The Cfb climate type is characterized by average temperatures not falling below -3°C in the coldest months, with precipitation evenly distributed throughout the year and maximum summer temperatures below 22°C. Over the last 30 years, at the Sarajevo-Bjelave station (630 m elevation), the average lowest temperature was recorded in January at 0.3°C, and the highest in July and August at 20.6°C. Average monthly precipitation is 78 mm, with the lowest in August (63.7 mm) and the highest in December (92 mm). In contrast, the Dfb zone features lower January temperatures (below -3°C), similar year-round precipitation patterns, and warm but not hot summers. At the Bjelašnica station (2,067 m), the annual average temperature is 2.0°C. January has the lowest monthly average (-6.6°C), while the highest is in August (11.5°C). Only July and August exceed 10°C in average monthly temperatures. Precipitation averages 93.7 mm monthly, peaking in November (113.5 mm) and dipping in January (78.5 mm) (FHMZ, 1992-2022).

Hydrologically, most of the canton (excluding the Rakitnica River, a Neretva tributary in the Adriatic Basin) belongs to the Black Sea drainage basin. The primary river system is the Bosna River, which originates in the central part of the canton and is fed by numerous tributaries.

The complex geological makeup, diverse topography, varied climate, and rich vegetation have led to a highly heterogeneous soil structure. Along river valleys, fluvisols dominate, along with luvisol–pseudogley complexes on Tertiary sediments and rendzina–dystric cambisol–pseudogley complexes on flysch formations. Higher elevations and slopes around the Sarajevo Basin, particularly in the south, southeast, and northeast, feature calcocambisols and mosaics of calcimelanosols on limestone substrates. In the north and in fragments of the south and southwest, mosaic patterns of calcimelanosols, calcocambisols, luvisols, and rendzina on limestone and dolomite prevail. The northwest and southwest areas feature dystric cambisols on acidic and silicate rocks (Burlica & Vukoper, 1983). This complex geotectonic, geological, geomorphological, climatological, hydrological, and pedological structure has fostered remarkable geodiversity and biodiversity within a relatively small area. Consequently, several protected natural areas have been recognized and designated within Canton Sarajevo. Currently, five areas are designated as protected: two natural monuments (Skakavac and Vrelo Bosne) and three protected landscapes (Bijambare, Trebević, and Bentbaša) (Tab. 1 & Fig 1).

Table 1. Protected Natural Areas in Canton Sarajevo (Federal Ministry of Environment and Tourism, 2024)

Protected Natural Area	**Year of Establishment**	**IUCN Category**	**Area (ha)**
Skakavac Natural Monument	2002 - 2010.	III	1430.70
Vrelo Bosne Natural Monument	2006.	III	603.44
Bijambare Protected Landscape	2006 - 2010.	V	497.0
Trebević Protected Landscape	2014.	V	400.2
Bentbaša Protected Landscape	2017.	V	160.9
TOTAL (ha)			**3092.24**

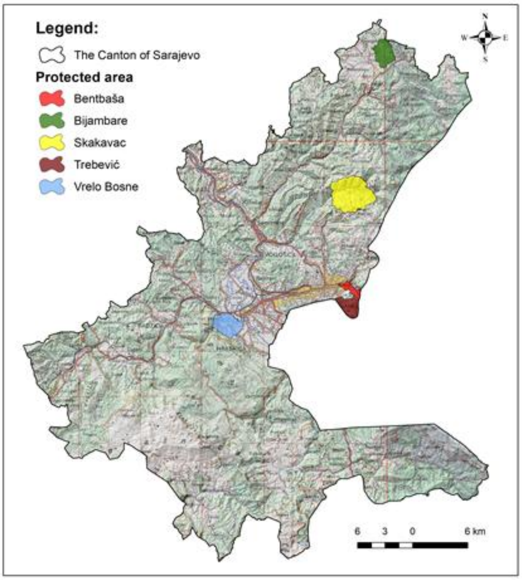

Figure 1. Protected Natural Areas of Sarajevo Canton
(Author: Hrelja, E., Source: Digital GIS Database, Faculty of Science, University of Sarajevo)

The total area of protected natural sites in Sarajevo Canton is 3,092.24 hectares (30.9 km²), which represents only 2.4% of the canton's total area. Both the number and extent of protected areas in Sarajevo Canton, and in Bosnia and Herzegovina as a whole, are below desirable levels. Only 18.7% of the protection targets set in the Bosnia and Herzegovina Spatial Plan for the period 1981–2000 have been achieved across different protection categories. An analysis of spatial planning documents at the entity level shows that the Federation of Bosnia and Herzegovina has planned to protect approximately 17% of its total territory, while Republika Srpska aims for around 15.5% (with a general target of 15–20% by 2025, according to amendments to the Spatial Plan of RS, 2013). This would bring national coverage to around 16% of Bosnia and Herzegovina's total area (Hrelja, 2022).

At the cantonal level, the management of protected areas is further defined through strategic development plans of regional self-government units, which elaborate on the development guidelines of entity-level documents. At the implementation level, these are governed by spatial plans. It is important to note that the management of all protected areas in the Sarajevo Canton is assigned to the Cantonal Public Institution for Protected Natural Areas (*Sl. Novine Kantona Sarajevo*, Br. 31, 2017).

Skakavac Natural Monument

The Skakavac Natural Monument is located approximately 12 km northeast of Sarajevo, on the slopes of Mount Ozren in the valley of the Perački Stream, which forms the core of the area's hydrographic network. The primary factor leading to its designation as a protected natural area is the Skakavac Waterfall. With a height of 98 meters, this waterfall serves as the nucleus of the protected zone and its central hydrographic and landscape feature. The water originates at the base of Bukovik peak, descending rapidly through a steep streambed until it reaches a gentle plateau, where the flow slows down and continues for several hundred meters before plunging off a vertical cliff of nearly 100 meters, forming one of the most beautiful waterfalls in Bosnia and Herzegovina (Dalmatin *et al*., 2008).

Orographically, the area is very heterogeneous, consisting of numerous valleys (gorges such as those of the Babin, Stublinski, Perački, Sušica, and Jasikovica streams), mountain peaks, ridges, and sinkholes, making it visually attractive and a popular destination for tourists. In addition to hydrological and geomorphological features, the significant heterogeneity of natural factors (geological foundation, soil types, relief, climate, and water networks) has led to the development of rich biodiversity and diverse habitats. The area is known for its high biological diversity, with more than 1,500 species of higher plants recorded, including several hundred species of medicinal, edible, aromatic, and vitamin-rich plants. The fauna is also highly diverse, with a high percentage of endemic species from the Dinaric, Balkan, and Southeastern European regions. The area also holds important cultural and historical value, with two stećak necropolises located nearby: a larger one near the village of Luka and a smaller one near Donji Močioci (Skakavac Natural Monument Management Plan 2011–2021).

Vrelo Bosne Natural Monument

Designated in 2006 as a Category III IUCN site, the Vrelo Bosne Natural Monument covers an area of 603.4 hectares. It is located in the southern part of the Sarajevo Basin, at the foot of Mount Igman. The main reason for its protection is the source springs of the Bosna River. In addition to the main spring, the area also contains multiple small streams and lakes, adding to its visual and ecological appeal.

The site is home to 131 tree species, 500 herbaceous plant species, over 50 species of medicinal and honey-bearing plants, more than 20 species of fungi and lichens, 102 species of microphytes, 19 mammal species, and 64 bird species, making it exceptionally important in terms of biodiversity (Dalmatin *et al*., 2008).

Endemic species of subterranean fauna have been recorded in the area of Vrelo Bosne, which further confirms its value in the context of biological diversity. Among them, two endemic species of crustaceans from the order Amphipoda: *Niphargus ilidzensis* and *Gammarus bosniacus*. The species *N. ilidzensis* was first described in 1922. and is characteristic of the underground waters of the wider area of Ilidža, including the Vrelo Bosne area. While *G. bosniacus*, described in the same year, represents another important element of the subterranean aquatic fauna of this locality. The presence of

these endemic species within the Vrelo Bosne protected area indicates a high degree of specificity and sensitivity of the ecosystem, and additionally emphasizes the need for its preservation through long-term monitoring and habitat protection (Plan upravljanja Spomenikom prirode „Vrelo Bosne“ 2020-2030).
Vrelo Bosne has long served as a popular recreational area for Sarajevo residents and spa visitors from Ilidža. It was appreciated during the Ottoman Empire, but its current layout was largely developed during the Austro-Hungarian period at the end of the 19th and beginning of the 20th century (Drešković *et al*., 2015). As a result of human activity within and around the area, numerous cultural and landscape features have been preserved. These include the Great Alley of Platanus and Chestnut Trees, the park grounds around Ilidža Spa and Stojčevac, the Roman Bridge water supply at Plandište, Austro-Hungarian villas, and the historic hotels of Ilidža, all of which contribute to the area's ecological, historical, and tourist value.

Bijambare Protected Landscape
Bijambare is located in the central Dinaric region of Bosnia and Herzegovina, approximately 25 km north of Sarajevo, between the villages of Nišići and Krivajevići in the northeastern part of the Crnorječka Plateau (Drešković & Đug, 2006; Hrelja *et al*., 2021). Geologically and tectonically, the Bijambare region has been shaped by a long and complex geological history, resulting in diverse formations. The Crnorječka Plateau is a depression located between mountain ranges, with elevations ranging from 915 to 1,044 meters. It is polygenetic in origin, formed through a combination of tectonic, neotectonic, and exogenic processes. Its surface features both fluvial and karstic terrain, depending on the bedrock.
The main geomorphological indicators justifying the area's protection are its characteristic karst formations, caves, sinkholes, dolines, and limestone ridges, especially well preserved in the western and northwestern parts. Notable formations include the Main (Middle) Bijambare Cave, the Upper Cave, Đurićina Cave, and the Lower Cave. A particularly valuable feature of the protected area is its peat bog ecosystem (Drešković *et al*., 2015). This soil type has formed on highly acidic, nutrient-poor substrates with low base saturation. Mosses of the genus *Sphagnum* dominate the vegetation, thriving in wet, acidic soils where peat forms from their decomposing remains. Peat formation began during the deglaciation period, when favorable climatic conditions emerged for intensified photosynthetic activity in the area’s herbaceous and woody vegetation (Barudanović *et al.,* 2017).

Trebević Protected Landscape
Established in 2014 as a Category V IUCN site, the Trebević Protected Landscape covers 400.2 hectares. It is located in the Trebević region of Sarajevo Canton and is particularly attractive to visitors from Sarajevo due to its favorable location and climate, especially in winter. The installation of a cable car and improved road infrastructure has made the site more accessible from the city’s historic core.

The area is characterized by significant geodiversity, particularly in terms of geological, geomorphological, and soil features. It also contains diverse flora and fauna, with more than 99 plant species, 14 fungal species, and over 26 bird species identified (Drešković *et al*., 2015). Its long history of human settlement and activity has left a rich cultural and historical legacy, including the Čolina Kapa astronomical observatory (Bistrik Fortress), remnants of Draguljac Fortress, Austro-Hungarian water supply systems, remains of the 1984 Olympic bobsled track, scenic viewpoints, mountain lodges, and more.

Bentbaša Protected Landscape

The Bentbaša Protected Landscape is the newest designated natural area in Sarajevo Canton, established in 2017 as a Category V IUCN site. It is located in the far eastern part of the canton and administratively belongs to the Stari Grad Municipality of Sarajevo. At just 160.9 hectares, it is the smallest protected area in the canton. Based on the number and distribution of natural values and the degree of environmental modification, two zones have been designated within the protected area: a core zone (nucleus) and a buffer zone (Kantonalni zavod za zaštitu kulturno-historijskog nasljeđa, 2020–2030). The protection area includes a canyon section of the Miljacka River, beginning several hundred meters upstream from Sarajevo City Hall. The region is known for its distinct physical and biological features (Dalmatin *et al*., 2008). These include the Miljacka watercourse with its canyon landscape, the valleys of its tributaries (Lapišnica and Mošćanica), various springs and wells, caves, and unique landforms such as the Babin Zub cliff. Due to ecological factors, the Miljacka River and its tributaries, over a 5–6 km stretch, are considered an endemic and relict refuge of Tertiary flora and fauna, centered around the Da Riva area and the confluence of the Mošćanica River (Dalmatin et al., 2008). The site has recorded high biodiversity with more than 160 plant species and 134 butterfly species (Kantonalna javna ustanova za zaštićena područja, 2025).

Bentbaša's proximity to Sarajevo’s historic center adds to its significance. In addition to natural values, the area is rich in cultural and recreational assets, including Goat’s Bridge (*Kozija Ćuprija* / The historic monument), Isa Bey’s Tekke and *Zavija* (The natural and architectural ensemble), Sheik’s Forest (*Šehova korija*), the Dervish Cave, and the *Ebu Hayat* Spring (“Spring of Life”). Recreational features include the Dariva promenade, bike trails, Ambassador’s Alley, and the former railway path.

Sampling and Species Analysis

Algal phytobenthos sampling for this study was conducted at selected locations within the protected areas of Bijambare and Vrelo Bosne. For the checklist preparation of other protected sites, data from relevant literature sources were utilized. Algal material from the Bijambare Protected Landscape was collected during the autumn season of 2022, while material from the Vrelo Bosne Natural Monument was collected in autumn 2023. All collected samples were transported and analyzed at the Laboratory for Systematics and Ecology of Algae, Fungi, and Lichens. Epipelon samples were taken from the uppermost layer of mud using a spoon or pipette aspirator. The collected material was preserved with 4% formalin. Laboratory processing of diatoms followed the methods described by Hustedt (1930).

Observations were performed using a Best Scope 2020 light microscope. Diatom species composition and their quantitative ratios were assessed using permanent slides examined at 1000x magnification. For each sample, a minimum of 400 valves were counted per slide. Diatom identification was based on the following references: Lange-Bertalot & Metzeltin (1996), Cantonati *et al*. (2017). The nomenclature of the identified algae was updated in accordance with Guiry (2024). Ecological data were analyzed using Omnidia software, version 6.0.8 (Lecointe *et al*., 1993).

In order to prepare a compartive overview of cyanobacteria and algae within the protected natural areas of Canton Sarajevo, the results of previously conducted research were used: Skakavac (Barudanović *et al*., 2014; 2016), Bijambare (Kapetanović *et al.,* 2011; Barudanović *et al.,* 2015; 2016 and Koštrebić, 2022) and Vrelo Bosne (Trožić-Borovac i Hafner, 2004; Barudanović *et al.,* 2016; Barudanović, 2020; Mašić i Macanović, 2020; Hadžić, 2023).

RESULTS AND DISCUSSION

The Canton Sarajevo, compared to other cantons in the Federation of Bosnia and Herzegovina, has the largest number of protected areas. The protected areas in KS are: Skakavac, Bijambare, Vrelo Bosne, Trebević and Bentbaša. Each of these protected areas is characterized by specific geomorphological, hydrological, pedological, and biological and ecological features. A distinctive feature of the declared protected areas is that they are centers of biological diversity in flora, fauna, and fungi. Certain groups of organisms within the protected areas are well-researched; however, for some other groups, no lists have been created yet, nor is there an organized database. A review of published studies on the protected areas in Canton Sarajevo indicates that the level of research on cyanobacteria and algae is least explored in the two newest protected areas: Trebević and Bentbaša, while the diversity of this group of organisms has been most extensively studied at Bijambare, Vrelo Bosne and Skakavac (Hrelja, 2017).

Taking into account the research conducted so far within the Protected Areas in Sarajevo Cantona, a total of 268 taxa of cyanobacteria and algae have been identified. The largest number of taxa was recorded in the Bijambare protected area (155), followed by the Vrelo Bosne (102) and Skakavac protected areas (66). There is no data on the number of cyanobacteria and algae taxa for the newly protected areas, Trebević and Bentbaša. The identified species belong to the following groups: Cyanophyta (28 taxa or 10.44%), Rhodophyta (1 taxon or 0.37%), Heterokontophyta (223 taxa or 83.20%), Dinophyta (1 taxon or 0.37%), and Chlorophyta (15 taxa or 5.59%). The results of the diversity research on cyanobacteria and algae within the protected areas in Sarajevo Canton are presented in tables, graphs, and original LM microphotographs (Tab. 2, 4, and Fig. 2).

Table 2. Percentage of cyanobacteria and algae taxa identified within the studied protected areas in Sarajevo Canton

Phylum	Classis	N	%
Cyanophyta	*Cyanophyceae*	28	10,44
Rhodophyta	*Florideophyceae*	1	0,37
Heterkontophyta	*Chrysophyceae*	1	0,37
	Xanthophyceae	2	9,74
	Bacillariophyceae	220	82,08
Dinophyta	*Dinophyceae*	1	0,37
Chlorophyta	*Chlorophyceae*	6	2,23
	Zygnematophyceae	8	2,98
	Ulvophyceae	1	0,37
	UKUPNO	**268**	**100**

When considering the distribution of identified taxa by classes, the results are as follows: *Cyanophycea*e (28 taxa or 10.44%), *Florideophyceae* (1 taxon or 0.37%), *Chrysophyceae* (1 taxon or 0.37%), *Xanthophyceae* (2 taxa or 0.74%), *Bacillariophyceae* (220 taxa or 82.08%), *Dinophyceae* (1 taxon or 0.37%), *Chlorophyceae* (6 taxa or 2.23%), *Ulvophyceae* (1 taxon or 0.37%) and *Zygnematophyceae* (8 taxa or 2.98%) (Fig. 3)

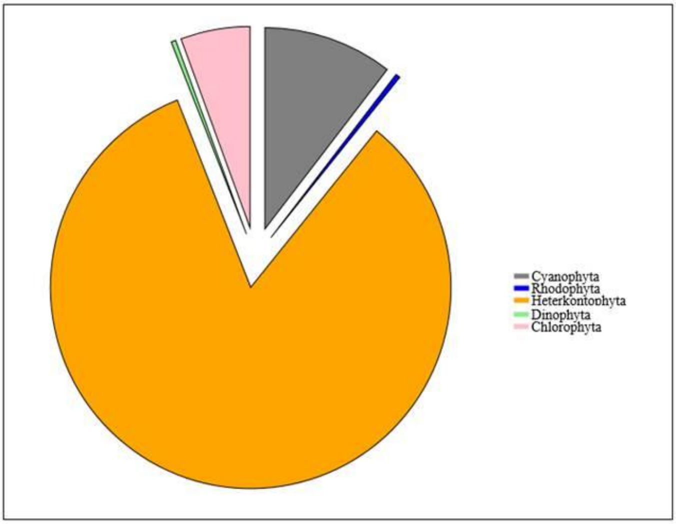

Figure 2. Percentage of cyanobacteria and algae species by class within the studied protected areas in Sarajevo Canton

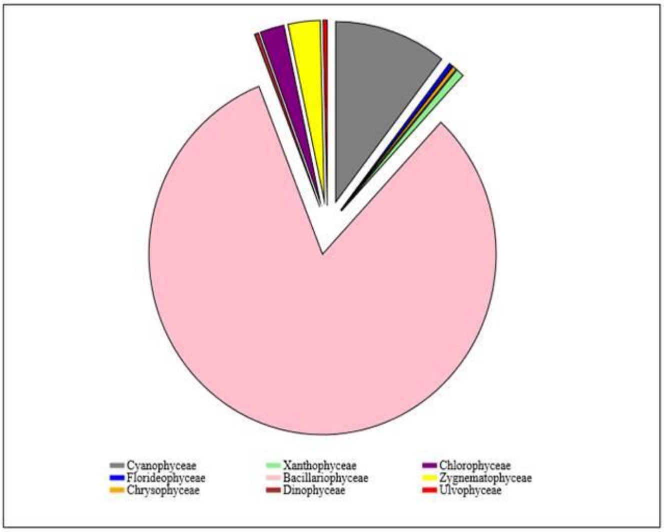

Figure 3. Percentage of cyanobacteria and algae species by class within the studied protected areas in Sarajevo Canton

By comparing the results obtained through research and those collected from published studies and reports, a checklist of cyanobacteria and algae in the Protected Areas of Sarajevo Canton has been prepared. The highest number of taxa was recorded in the Bijambare Protected Area (155 taxa), followed by the Vrelo Bosne Protected Area (102 taxa), while the Skakavac Protected Area recorded the lowest number of species (66 taxa) of cyanobacteria and algae. A comparative overview of the diversity of cyanobacteria and algae is presented in tables and graphs (Tab. 3 and Fig. 4).

Table 3. Comparative Overview of Cyanobacteria and Algae Diversity within the Studied Protected Areas in Sarajevo Canton

Protected area in Canton Sarajevo	**Cyanophyceae**	**Florideopgyceae**	**Chrysophyceae**	**Xanthophyceae**	**Bacillariophyceae**	**Dinophyceae**	**Chlorophyceae**	**Zygnematophyceae**	**Ulvophyceae**	**TOTAL**
Bijambare	12	.	.	.	139	.	2	2	.	155
Vrelo Bosne	19	1	.	2	73	.	3	4	.	102
Skakavac	.	.	1	.	59	1	1	3	1	66
Trebević	.	.	.	.	.	.	.	.	.	N/a
Bentbaša	.	.	.	.	.	.	.	.	.	N/a

Within the Bijambare Protected Area, a total of 155 taxa of cyanobacteria and algae have been identified to date. The research within this protected area covered almost all wet habitats, primarily peat bog ecosystems and cavernicolous habitats. The identified taxa belong to the following classes: *Cyanophyceae* (12), *Bacillariophyceae* (139), *Chlorophyceae* (2) and *Zygnematophyceae* (2) (Tab. 3; Fig. 3, 4).

Within the Vrelo Bosne Protected Area, a total of 102 taxa of cyanobacteria and algae have been identified to date. The research within this protected area covered springs, streams, and pools. The identified taxa belong to the following classes: *Cyanophyceae* (19), *Florideophyceae* (1), *Xanthophyceae* (2), *Bacillariophyceae* (73), *Chlorophyceae* (3), and *Zygnematophyceae* (4) (Tab. 3; Fig. 3, 4).

Within the Skakavac Protected Area, a total of 66 taxa of cyanobacteria and algae have been identified to date. The research within this protected area covered various wet habitats, but the primary focus was on the Skakavac stream below the waterfall of the same name. The identified taxa belong to the following classes: *Chrysophyceae* (1), *Bacillariophyceae* (59), *Dinophyceae* (1), *Chlorophyceae* (1), *Ulvophyceae* (1) and *Zygnematophyceae* (3) (Tab. 3; Fig. 3, 4).

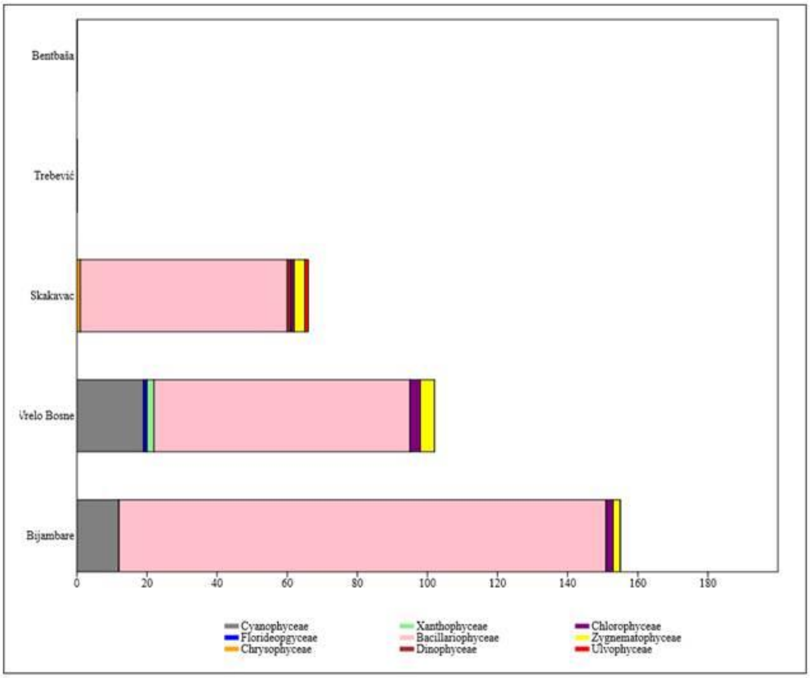

Figure 4. Comparative Overview of Cyanobacteria and Algae Diversity within the Studied Protected Areas in Sarajevo Canton

Data on the diversity of cyanobacteria and algae for the Trebević and Bentbaša protected areas have not been recorded in existing documents. In this regard, future detailed research is essential to gather data that can be used to prepare a digital database of cyanobacteria and algae, as well as their distribution within the protected areas. Given all the information provided, it can be concluded that the protected areas in Sarajevo Canton are characterized by a high degree of cyanobacteria and algae diversity. Considering that two protected areas have not been studied in terms of the diversity of this group of organisms, it is estimated that the number of species is likely even higher. Since cyanobacteria and algae are excellent bioindicators of aquatic ecosystems, it is necessary to include them in planning documents for continuous monitoring of these ecosystems.

Table 4. Comparative Overview of Cyanobacteria and Algae Diversity within the studied natural protected areas in Canton Sarajevo

ID	Diversity of cyanobacteria and algae in Protecte Areas of Canton Sarajevo	Odjel	Klasa	Skakavac[1, 2]	Bijambare[2,3,4,8]	Vrelo Bosne[2,5,6,7]	Trebević	Bentbaša	Ukupno
1.	*Achnanthidium minutissimum* (Kütz.) Czarn.	Heterokontophyta	*Bacillariophyceae*	1	1	1	0	0	3
2.	*Amphora ovalis* (Kützing) Kützing var. *ovalis*	Heterokontophyta	*Bacillariophyceae*	1	1	1	0	0	3
3.	*Cocconeis pediculus* Ehrenberg	Heterokontophyta	*Bacillariophyceae*	1	1	1	0	0	3
4.	*Denticula tenuis* Kütz.	Heterokontophyta	*Bacillariophyceae*	1	1	1	0	0	3
5.	*Diatoma vulgaris* Bory	Heterokontophyta	*Bacillariophyceae*	1	1	1	0	0	3
6.	*Eunotia minor* (Kützing) Grunow in Van Herck	Heterokontophyta	*Bacillariophyceae*	1	1	1	0	0	3
7.	*Melosira varians* Agardh	Heterokontophyta	*Bacillariophyceae*	1	1	1	0	0	3
8.	*Navicula sp.*	Heterokontophyta	*Bacillariophyceae*	1	1	1	0	0	3
9.	*Pinnularia viridis* (Nitzsch.) Ehrenb.	Heterokontophyta	*Bacillariophyceae*	1	1	1	0	0	3
10.	*Achnanthes sp.*	Heterokontophyta	*Bacillariophyceae*	0	1	1	0	0	2
12.	*Caloneis silicula* (Ehr.) Cleve	Heterokontophyta	*Bacillariophyceae*	1	0	1	0	0	2
13.	*Campylodiscus hibernicus* Ehrenberg	Heterokontophyta	*Bacillariophyceae*	1	0	1	0	0	2
14.	*Chroococcus minutus* (Kützing) Nägeli	Cyanophyta	*Cyanophyceae*	0	1	1	0	0	2
15.	*Cocconeis placentula* var. *lineata* (Ehrenberg) Van Heurck	Heterokontophyta	*Bacillariophyceae*	1	0	1	0	0	2
16.	*Cyanothece aeruginosa* (Nägeli) Komárek	Cyanophyta	*Cyanophyceae*	0	1	1	0	0	2
20.	*Diatoma hyemalis* (hiemale) (Roth) Heiberg	Heterokontophyta	*Bacillariophyceae*	1	0	1	0	0	2
21.	*Diatoma mesodon* (Ehrenberg) Kützing	Heterokontophyta	*Bacillariophyceae*	1	0	1	0	0	2

22.	*Diploneis oblongella* (Naegeli) Cleve-Euler	Heterokontophyta	*Bacillariophyceae*	1	0	1	0	0	2
23.	*Ellerbeckia arenaria* (D.Moore ex Ralfs) Dorofeyuk & Kulikovskiy	Heterokontophyta	*Bacillariophyceae*	1	0	1	0	0	2
25.	*Encyonema ventricosum* (Kützing) Grunow in Schmidt & al.	Heterokontophyta	*Bacillariophyceae*	0	1	1	0	0	2
27.	*Epithemia adnata* (Kützing) Brébisson	Heterokontophyta	*Bacillariophyceae*	0	1	1	0	0	2
32.	*Gomphonema olivaceum* (Hornemann) Brébisson var. olivaceum	Heterokontophyta	*Bacillariophyceae*	1	0	1	0	0	2
33.	*Gomphonema sp.*	Heterokontophyta	*Bacillariophyceae*	1	0	1	0	0	2
34.	*Gomphonema subclavatum* Grunow	Heterokontophyta	*Bacillariophyceae*	0	1	1	0	0	2
35.	*Hantzschia amphioxys* (Ehr.) Grunow in Cleve et Grunow	Heterokontophyta	*Bacillariophyceae*	0	1	1	0	0	2
36.	*Navicula lanceolata* (Agardh) Ehrenberg	Heterokontophyta	*Bacillariophyceae*	1	0	1	0	0	2
38.	*Navicula tripunctata* (O.F.Mütller) Bory	Heterokontophyta	*Bacillariophyceae*	1	0	1	0	0	2
39.	*Nitzschia sp*.	Heterokontophyta	*Bacillariophyceae*	1	0	1	0	0	2
40.	*Oscillatoria sp.*	Cyanophyta	*Cyanophyceae*	0	1	1	0	0	2
42.	*Spirogyra sp.*	Chlorophyta	*Conjugatophyceae*	1	0	1	0	0	2
43.	*Stauroneis smithii* Grunow	Heterokontophyta	*Bacillariophyceae*	1	0	1	0	0	2
44.	*Stauroneis phoenicenteron* (Nitzsch) Ehrenberg	Heterokontophyta	*Bacillariophyceae*	1	0	1	0	0	2
45.	*Ulnaria ulna* (Nitzsch.) Compere	Heterokontophyta	*Bacillariophyceae*	1	0	1	0	0	2
46.	*Achnanthes exigua* Grunow	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
47.	*Achnanthidium pyrenaicum* (Hustedt) H.Kobayasi	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
50.	*Amphipleura pellucida* (Kützing) Kützing	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1

51.	*Amphora minuta*	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
54.	*Audouinella violacea* (Kützing) Hamel	Rhodophyta	*Florideophyceae*	0	0	1	0	0	1
56.	*Aulocoseira ambigua* (Grunow) Simonsen	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
57.	*Caloneis alpestris* (Grunow) Cleve	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
65.	*Chamaesiphon confervicola*	Cyanophyta	*Cyanophyceae*	0	0	1	0	0	1
66.	*Chamaesiphon incrustans* Grunow in Rabehorst	Cyanophyta	*Cyanophyceae*	0	0	1	0	0	1
74.	*Closterium acerosum* Ehrenberg ex Ralfs	Chlorophyta	*Conjugatophyceae*	0	0	1	0	0	1
75.	*Closterium ehrenbergii* Meneghini ex Ralfs	Chlorophyta	*Conjugatophyceae*	0	0	1	0	0	1
78.	*Cocconeis placentula* Ehrenberg	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
81.	*Craticula cuspidata* Kützing	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
83.	*Cymatopleura elliptica*	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
85.	*Cymatopleura solea* var. apiculata	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
87.	*Cymbella helvetica* Kützing	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
89.	*Cymbella aspera* (Ehrenberg) Cleve	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
90.	*Cymbella cistula* (Ehrenberg) O.Kirchner	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
91.	*Cymbella lanceolata* C.Agardh	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
92.	*Denticula elegans* Kützing	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
93.	*Denticula kuetzingii* Grunow 1862	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
101.	*Diploneis ovalis* (Hilse) Cleve	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1

102.	*Discostella stelligera* (Cleve & Grunow) Houk & Klee	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
109.	*Encyonema minutum* (Hilse) D.G.Mann	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
113.	*Epithemia sp.*	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
114.	*Epithemia argus* (Ehrenberg) Kützing	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
125.	*Eunotia minor* (Kützing) Grunow	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
130.	*Fragilaria vaucheriae* (Kütz.) Petersen	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
141.	*Gomphonema micropus* Kützing	Heterokontophyta	*Bacillariophyceae*	0	1	1	0	0	2
142.	*Gomphonema minuscula*	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
144.	*Gomphonema acuminatum* Ehrenberg	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
145.	*Gomphonema olivaceum* (Hornemann) Ehrenberg	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
147.	*Gyrosigma obtusatum* (Sullivan & Wormley) Boyer	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
148.	*Gyrosigma acuminatum* (Kützing) Rabenhorst 1853	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
149.	*Gyrosigma scalproides* (Rabenhorst) Cleve 1894	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
154.	*Homoeothrix varians* Geitler	Cyanophyta	*Cyanophyceae*	0	0	1	0	0	1
163.	*Lyngbia martensiana* (Meneghini) Gomont	Cyanophyta	*Cyanophyceae*	0	0	1	0	0	1
167.	*Microspora sp.*	Chlorophyta	*Chlorophyceae*	0	0	1	0	0	1
168.	*Mougeotia genuflexa* (Roth) C.Agardh	Chlorophyta	*Conjugatophyceae*	0	0	1	0	0	1
174.	*Navicula veneta* Kütz.	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
175.	*Navicula lanceolata* Ehrenberg	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1

176.	*Navicula radiosa* Kützing	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
188.	*Nitzschia sigma*	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
191.	*Nitzschia dissipata* (Kützing) Rabenhorst	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
192.	*Nitzschia fonticola* (Grunow) Grunow	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
193.	*Nitzschia recta* Hantzsch ex Rabenhorst	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
198.	*Oedogonium fonticola* A.Braun ex Hirn	Chlorophyta	*Chlorophyceae*	0	0	1	0	0	1
199.	*Oedogonium sp.*	Chlorophyta	*Chlorophyceae*	0	0	1	0	0	1
202.	*Oscillatoria princeps* Vaucher ex Gomont	Cyanophyta	*Cyanophyceae*	0	0	1	0	0	1
206.	*Phormidium favosum* Gomont	Cyanophyta	*Cyanophyceae*	0	0	1	0	0	1
207.	*Phormidium inundatum* (Kützing) Gomont	Cyanophyta	*Cyanophyceae*	0	0	1	0	0	1
208.	*Phormidium papyraceum* Gomont ex Gomont	Cyanophyta	*Cyanophyceae*	0	0	1	0	0	1
209.	*Phormidium retzii* (Meneghini) Gomont	Cyanophyta	*Cyanophyceae*	0	0	1	0	0	1
210.	*Phormidium sp.*	Cyanophyta	*Cyanophyceae*	0	0	1	0	0	1
223.	*Pinnularia rupestris* Hantzsch in Rabenhorst	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
231.	*Placoneis sp.*	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
233.	*Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
234.	*Plantktolyngbya sp.*	Cyanophyta	*Cyanophyceae*	0	0	1	0	0	1
235.	*Plectonema tomasinianum* (Bornet 1889) Gomont	Cyanophyta	*Cyanophyceae*	0	0	1	0	0	1
236.	*Plectonema wollei* Farlow ex Gomont	Cyanophyta	*Cyanophyceae*	0	0	1	0	0	1

237.	*Pseudoanabaena catenata*	Cyanophyta	*Cyanophyceae*	0	0	1	0	0	1
239.	*Rhoicosphaenia abbreviata*	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
247.	*Sellaphora sp.*	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
249.	*Shizothrix fasciculata* (Nägeli in Kützing) Gomont	Cyanophyta	*Cyanophyceae*	0	0	1	0	0	1
250.	*Spirulina sp.*	Cyanophyta	*Cyanophyceae*	0	0	1	0	0	1
262.	*Surirella linearis* W.M.Smith in Schmidt & al.	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
263.	*Surirella sp.*	Heterokontophyta	*Bacillariophyceae*	0	0	1	0	0	1
267.	*Tribonema viridae* Pascher	Heterokontophyta	*Xanthophyceae*	0	0	1	0	0	1
268.	*Vaucheria sp.*	Heterokontophyta	*Xanthophyceae*	0	0	1	0	0	1

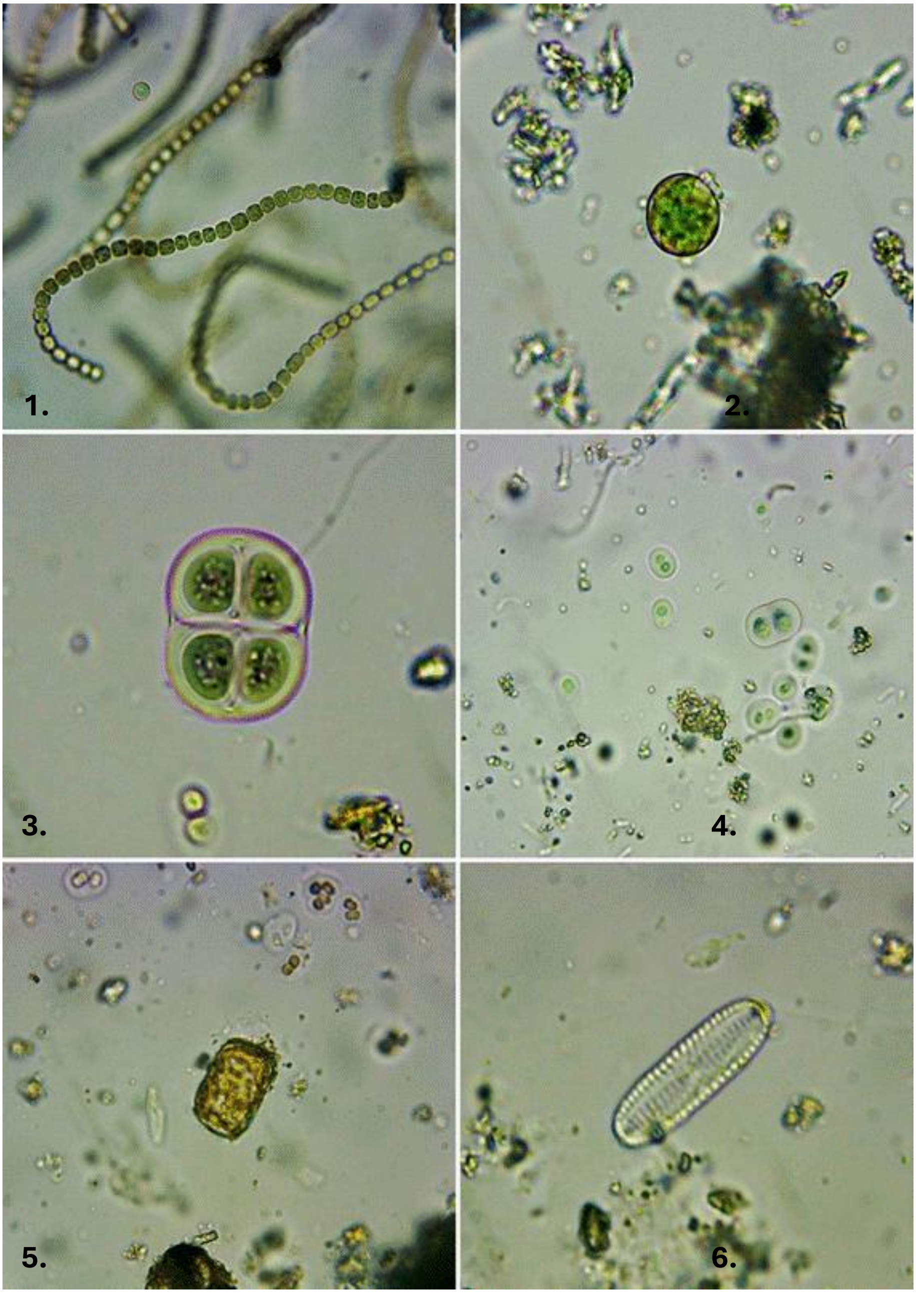

Plate A. Selected taxa of cyanobacteria and algae identified in the Protected areas in Canton Sarajevo: *1. Nostoc commune, 2. Chlorella vulgaris, 3. Chroococcus turgidus, 4. Chroococcus minutus, 5. Orthoseira roeseana, 6. Pinnularia borealis*

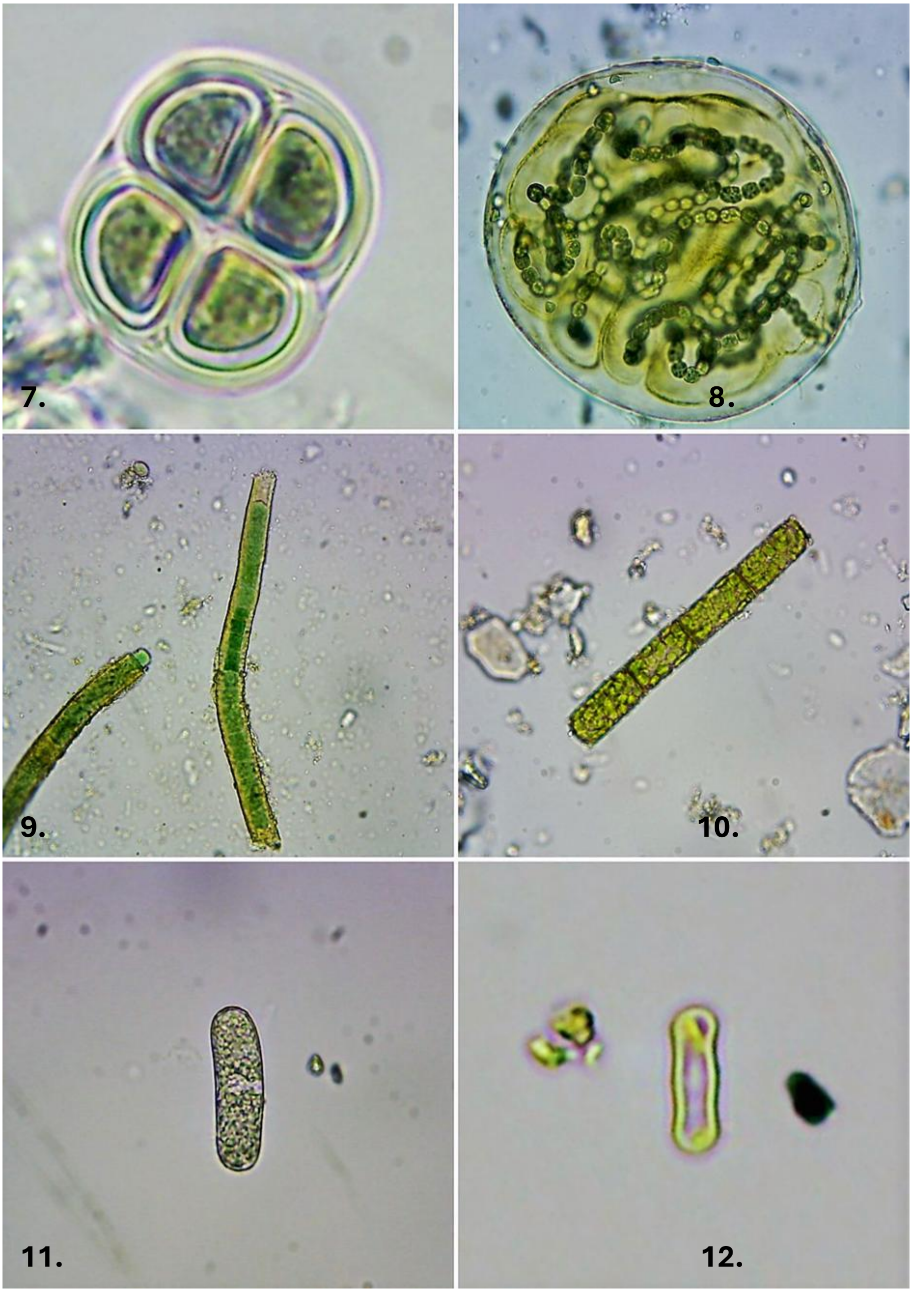

Plate B. Selected taxa of cyanobacteria and algae identified in the Protected areas in Canton Sarajevo: *7. Chroococcus turgidus, 8. Nostoc commune, 9. Phormidium calcareum, 10. Orthoseira dendroteres, 11. Cyanothece aeruginosa, 12. Humidophila contenta*

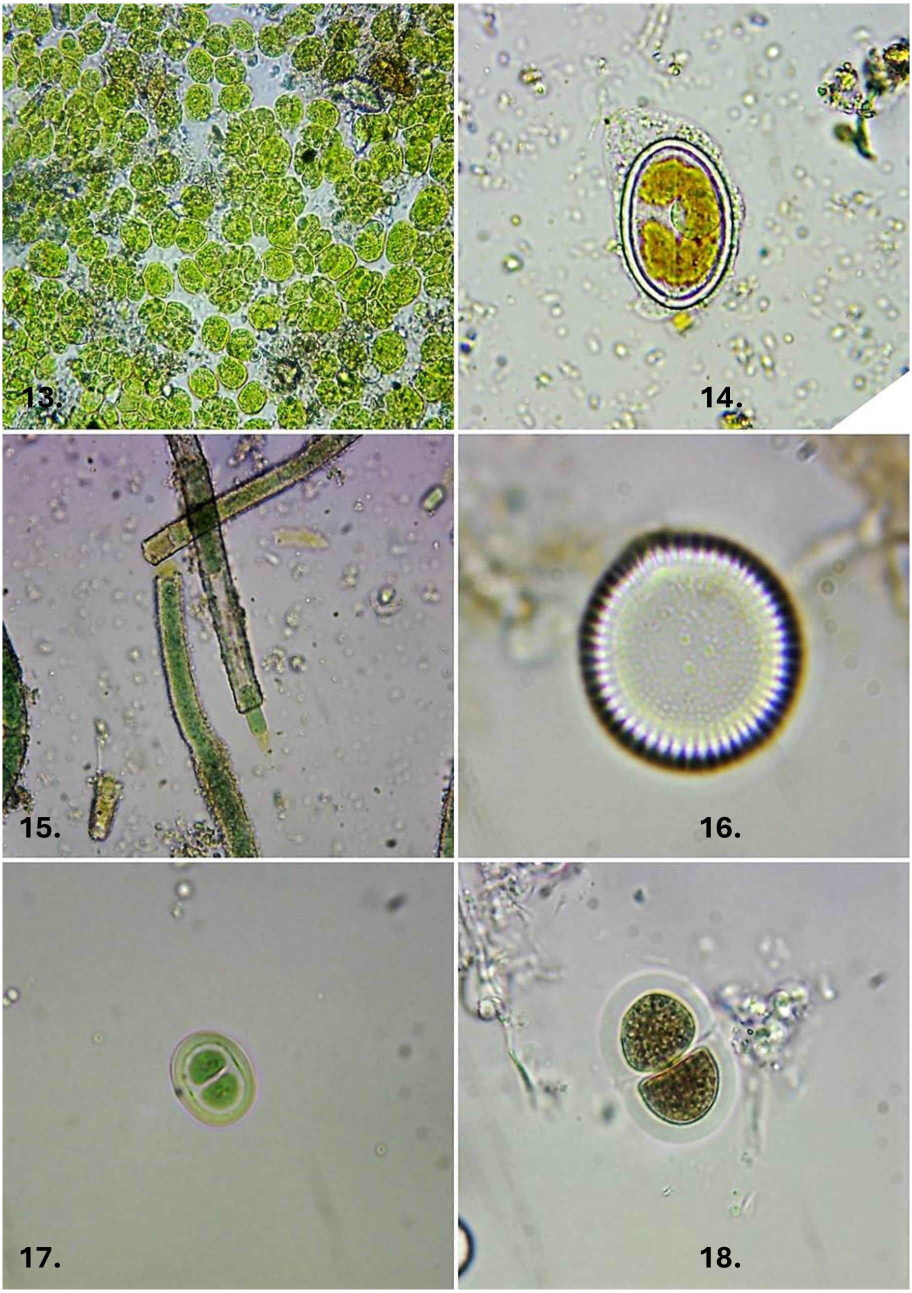

Plate C. Selected taxa of cyanobacteria and algae identified in the Protected areas in Canton Sarajevo: *13. Chlorella vulgaris, 14. Cocconeis pediculus, 15. Phormidium calcareum, 16. Orthoseira roeseana, 17. Chroococcus minutus, 18. Chroococcus turgidus*

RESULTS AND DISCUSSION

The study of cyanobacteria and algae in protected areas across Europe varies depending on specific locations and the research efforts made in those areas. Europe is rich in diverse protected areas, such as national parks, biosphere reserves, strict nature reserves, and other regions with special protection statuses that contribute to maintaining ecosystems. Additionally, the study of underwater organisms, such as marine invertebrates, fish, and marine algae, can be challenging due to access and logistical issues (Ismail & Goeden, 2022).

Many research institutions, universities, and organizations in Europe focus on water ecosystems, including the study of cyanobacteria and algae. These researchers often collaborate with local authorities and nature protection agencies to collect data on the presence, diversity, ecology, and water quality in protected areas. Studies frequently focus on monitoring water quality and assessing ecological status using bioindicator organisms, including cyanobacteria and algae. Monitoring cyanobacteria is particularly important due to the potential for toxic blooms in water bodies, which can pose risks to the environment and human health (Benayache *et al*., 2019).

Cyanobacteria and algae represent an essential component of aquatic ecosystems in protected areas throughout Europe. The study of cyanobacteria and algae in protected areas has several critical aspects. Firstly, it provides information about the state of aquatic ecosystems and any potential changes in their biological diversity. This is crucial for monitoring and conserving the health of aquatic ecosystems, especially in the context of climate change and anthropogenic impacts. Secondly, the study of cyanobacteria is important for assessing water quality and safety for human use. Identifying potentially toxic species of cyanobacteria and monitoring their presence allows for preventive measures and risk management of toxic blooms. The study of cyanobacteria and algae in protected areas is of significant importance for the continuous monitoring of the ecological status of surface and groundwater, the conservation of sensitive microhabitats and the species contained therein, and their potential ecological restoration. Numerous aquatic microhabitats within protected areas provide valuable ecosystem services that improve the quality of life for humans. In this regard, it is essential to protect them from anthropogenic impacts and various tourist activities, utilizing various mechanisms and tools such as the Convention on Biological Diversity (CBD) to preserve biodiversity in Europe (Li *et al*., 2023; Pergl *et al.,* 2020).

To assess the diversity of cyanobacteria and algae in protected areas across Europe, databases and the scientific papers contained within them were analyzed. Papers focusing primarily on this group of organisms were selected. In this regard, studies on cyanobacteria and algae in the following European countries were analyzed: Ukraine (Polesian Nature Reserve, Podilsky Tovtry National Park, and the Nature Park of Priazov), Czech Republic (Soos Nature Reserve), Spain (Corrubedo National Park), Turkey (Artabel Lakes Nature Park), and Bulgaria (Bulgarka National Park) [Bren *et al*. (2022), Atici, 2018, Stoyanov *et al*. (2016), Kapustin, 2014, Momeu *et al*. (2012), Vinogradova *et al*. (2009), Gross *et al*. (2002), and Calvo *et al.* (1999)].

In the study investigating the Polesian Nature Reserve, the author (Kapustin, 2014) examined algal biodiversity and identified a total of 707 taxa. The presence of many rare and endangered species of algae was noted. Notably, seven species were listed in the Red Book of Ukraine. The dominant groups in terms of species number were Chlorophyta (160), Bacillariophyta (159), Streptophyta (144), and Euglenophyta (111), while others appeared in smaller numbers.
In addition to this protected area, Ukraine also has the Nature Park of Priazov, where research by Bren *et al*. (2022) indicates the existence of 11 classes, 32 orders, 61 families, and 92 genera. The greatest species richness at the departmental level was observed in Cyanophyta (43.8% of the total species), Bacillariophyta (32.0%), and Chlorophyta (20.9%). The leading algal orders in the studied habitats were Oscillatoriales and Synechococcales (20 species). The most diverse families were *Oscillatoriaceae* (13 taxa), *Nostocaceae*, *Leptolyngbyaceae*, *Naviculaceae* (8 taxa), *Microcoleaceae* and *Bacillariaceae* (7 taxa).
The Soos Nature Reserve, located in the westernmost part of the Czech Republic, has a total of 35 species. The study (Gross *et al*., 2002) highlights the area's interesting pH range of 1.6 to 2.6, with very little algal biomass, which is mostly composed of *Chlamydomonas acidophila* (Chlorophyta, Chlamydomonadales), *Lepocinclis teres*, *Euglena mutabilis* (Euglenophyta, Euglenales), and *Eunotia exiqua*, among others.
In a selected study of algae in the Artabel Lakes Nature Park in Turkey, the author (Atici, 2018) identified a total of 98 species belonging to the departments: Bacillariophyta, Chlorophyta, Cyanobacteria, Euglenophyta, Pyrrophyta, and Cryptophyta. The study emphasized that the samples from this Nature Park were analyzed from both planktonic and benthic samples from 18 freshwater lakes.
The results of studies on benthic marine algae from the saline marshes of Corrubedo National Park on the Iberian Peninsula (Calvo *et al*., 1999) indicate a total of 84 taxa. A total of 84 species were identified, including 49 Cyanophyta, 6 Rhodophyta, 10 Heterokontophyta, and 19 Chlorophyta. Many taxa are of biogeographical interest. *Vaucheria arcassonensis*, *V. intermedia*, and *Wittrockiella paradoxa* are new records from the Iberian Peninsula. Cyanophyta and Chlorophyta together comprise 81% of the reported species, with chlorophyte algae and *Vaucheria sp.* making up the most widespread population.
In five karstic speleo objects of the National Park of Podilsky Tovtry, the authors (Vinogradova *et al*., 2009) recorded 86 species. These belong to Cyanoprokaryota (35 species), Chlorophyta (35), Bacillariophyta (7), Xanthophyta (4), Streptophyta (3), and Eustigmatophyta (2). A total of 56 species were cited for the first time in this National Park, and eight species were new to Ukraine. In the caves, 67 species were recorded, with green algae (49-58%) dominating, and cyanoprokaryotes making up 11-29%.
In Bulgaria, in the Bulgarka National Park, the authors (Stoyanov *et al*., 2016) described 194 species belonging to 11 departments. Some of the species present include *Chroococcus turgidus*, *Cylindrospermum muscicola*, *Geitlerinema amphibium*, *Gloeothece fuscolutea*, *Heteroleibleinia rigidula*, and *Jaaginema gracile*, among others

Research on cyanobacteria and algae in protected areas of Bosnia and Herzegovina has not been extensively conducted. Sporadic data can be found in works by Kamberović *et al.* (2023), Stanić-Koštroman *et al.* (2021), Kamberović *et al.* (2021), Kamberović *et al.* (2019), Kamberović *et al.* (2016), Kapetanović *et al.* (2011), Kapetanović *et al.* (2007), Barudanović *et al.* (2025), Šovran *et al.* (2024), Barudanović *et al.* (2019), Mašić (2020), Mašić & Barudanović (2020), and Mašić *et al.* (2019). Unfortunately, there is currently no unified database listing the identified species in protected areas of Bosnia and Herzegovina or a display of their distribution across different aquatic ecosystems and wetland habitats. By comparing the composition of cyanobacteria and algae in protected areas of Sarajevo Canton with protected areas across Europe, it can be concluded that the number of taxa is high and characterized by specific diversity. Therefore, future efforts should focus on conducting fundamental research and expanding the list of cyanobacteria and algae in protected areas of Sarajevo Canton, as well as establishing a unified digital database on the diversity of cyanobacteria and algae in protected areas of Bosnia and Herzegovina.

CONCLUSIONS

The number and area of protected natural areas in Bosnia and Herzegovina (B&H), as well as in each of its administrative units, are not at a satisfactory level. B&H has a significantly lower level of protection compared to IUCN recommendations. Additionally, a significant number of protected areas are isolated without established adequate management mechanisms. The low level of protection is aligned with the country's various political and economic processes, which tend to prioritize resource exploitation over conservation. The importance of geodiversity and biodiversity is not viewed from the perspective of the ecosystem services that these protected areas provide, nor from the needs that can be met within them.

Protected natural areas, through their ecosystem services, are highly significant for society and the economy at local, regional, national, and global levels. They play a vital role in addressing the challenges of climate change, maintaining water quality, preserving natural pollinators, conserving biodiversity, geodiversity, and landscapes, as well as supporting tourism and recreation.

The study on the diversity of cyanobacteria and algae in the protected areas of Sarajevo Canton has shown that these habitats provide exceptionally favorable ecological conditions for their development. Compared to available scientific data from B&H and Europe, these areas facilitate the formation of stable communities of these organisms. However, there is still insufficient data for the newly protected areas, Bentbaša and Trebević, indicating a need for further research in the future.

Given that there is no unified biodiversity database for cyanobacteria and algae in B&H, its urgent establishment is necessary. Such a database would not only enable systematic monitoring and better understanding of this group of organisms but also play a crucial role in the long-term conservation of aquatic and wetland habitats within protected areas. Cyanobacteria and algae, being highly sensitive organisms, can serve as early indicators of ecological changes, helping to enable timely responses and more efficient natural resource management within protected areas. Their continuous monitoring and protection would contribute to the preservation of these valuable ecosystems for future generations.

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DOPRINOS POZNAVANJU RAZNOLIKOSTI CIJANOBAKTERIJA I ALGI U ODABRANIM ZAŠTIĆENIM PODRUČJIMA KANTONA SARAJEVO

Sažetak

Kanton Sarajevo ima najveći broj zaštićenih prirodnih područja među ostalim kantonima u Federaciji Bosne i Hercegovine, uključujući spomenike prirode i zaštićene krajolike: Skakavac, Bijambare, Vrelo Bosne, Trebević i Bentbaša. Svako od ovih područja karakteriziraju specifične geomorfološke, hidrološke, pedološke, biološke i ekološke značajke, služeći kao centri biološke raznolikosti za floru, faunu i gljive. Ovaj rad pruža uvid u raznolikost cijanobakterija i algi unutar odabranih zaštićenih područja Kantona Sarajevo. Do danas je unutar tih područja identificirano 268 taksona cijanobakterija i algi. Bijambare se mogu pohvaliti najvećim brojem vrsta (155), a slijede Vrelo Bosne (66) i Skakavac (66). Podaci o raznolikosti ovih organizama u Bentbaši i Trebeviću trenutno nisu dostupni, što naglašava potrebu za detaljnijim i

sistemskim istraživanjem na tim područjima. Osim uspostavljanja popisa cijanobakterija i algi, kontinuirano praćenje njihovih staništa u zaštićenim područjima Kantona Sarajevo je ključno. S obzirom na to da su cijanobakterije i alge izvrsni bioindikatori vodenih ekosistema, njihovo uključivanje u planske dokumente za kontinuirano praćenje ključan je korak u očuvanju ovih vrijednih područja.

Ključne riječi: *alge, bioraznolikost, cijanobakterije, praćenje, zaštićena područja*

PERCEPCIJA I ODGOVOR HRVATSKIH POLJOPRIVREDNIKA NA KLIMATSKE PROMJENE-PRELIMINARNI REZULTATI ISTRAŽIVANJA*

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Originalni naučni rad - *Original scientific paper*

Sažetak

Klimatske promjene predstavljaju ozbiljan izazov za poljoprivredni sektor, zahtijevajući razumijevanje percepcija poljoprivrednika i njihovih strategija prilagodbe za učinkovite javne politike. Istraživanje na prigodnom uzorku od 100 nositelja obiteljskih gospodarstava u Hrvatskoj pokazalo je visoku svijest o prijetnjama ekosustavima, bioraznolikosti i sigurnosti hrane. Poljoprivrednici naglašavaju potrebu za konkretnijim djelovanjem vlade i primjećuju značajne ekonomske gubitke.
Postoji konsenzus o važnosti klimatski prihvatljivih praksi, ali i zabrinutost zbog nedovoljne svijesti o rizicima. Najčešće strategije prilagodbe uključuju zelenu gnojidbu, otporne usjeve i navodnjavanje. Prihodi i obrazovanje utječu na usvajanje prilagodbi, ukazujući na socioekonomske faktore kao ključne u adaptaciji.
Poljoprivrednici su svjesni da klimatske promjene utječu na njih, te da je potrebno djelovati. Istraživanje je pokazalo da je potrebno raditi na edukaciji poljoprivrednika o rizicima klimatskih promjena, te ih poticati na primjenu klimatski prihvatljivih praksi. Također, potrebno je da vlada donese konkretnije politike koje će pomoći poljoprivrednicima u prilagodbi na klimatske promjene. Preporučuje se daljnje istraživanje na reprezentativnijem uzorku za generalizaciju rezultata. To će omogućiti sveobuhvatnije razumijevanje izazova s kojima se suočavaju poljoprivrednici u Hrvatskoj i doprinijeti razvoju učinkovitih strategija prilagodbe na klimatske promjene.

Ključne riječi: *klimatske promjene, poljoprivrednici, percepcija, prilagodba, strategije*

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Percepcija poljoprivrednika o klimatskim promjenama značajno utječe na svakodnevnu poljoprivrednu praksu i procese donošenja poslovnih odluka. S obzirom na to da se promjene klime manifestiraju kroz promjene u utvrđenim vremenskim obrascima te povećanu učestalost ekstremnih vremenskih događaja, važno je razumjeti kako poljoprivrednici tumače te promjene u svrhu donošenja učinkovite strategije prilagodbe za ublažavanje nepovoljnih utjecaja.

Percepcija ne samo da igra ključnu ulogu u određivanju specifičnih izbora koje poljoprivrednici donose u vezi s odabirom usjeva, metodama navodnjavanja i cjelokupnim upravljanjem gospodarstvom, već također i temeljno utječe na stupanj do kojeg su ti poljoprivrednici spremni usvojiti adaptivna ponašanja (eng. *adaptive behavior*) za poboljšanje njihove otpornost (van Valkengoed i Sted, 2019).

Nadalje, razne prepreke (Ricart i sur., 2023; Yadav i sur., 2023) s kojima se poljoprivrednici susreću, kao što su nedovoljna razina znanja, previsoki troškovi povezani s uvođenjem novih praksi (tehnologija) i neadekvatna institucionalna potpora, mogu značajno ograničiti njihovu sposobnost da prevedu svijest o klimatskim promjenama u postupke koji mogu doprinijeti njihovoj poljoprivrednoj proizvodnji. Stoga je ključno razviti i provesti djelotvorne javne politike (Amruddin, 2024) za rješavanje ovih prepreka, uzimajući u obzir različite percepcije i specifične potrebe poljoprivrednika, s krajnjim ciljem jačanja njihove otpornosti i promicanja održivih poljoprivrednih praksi koje mogu izdržati izazove koje donosi promjena klime.

Rezultati istraživanja (Raman i sur., 2024) pokazuju da su poljoprivrednici svjesni klimatskih promjena i njihovih potencijalnih utjecaja, a da njihove percepcije oblikuju između ostalog i socio-demografski čimbenici (dob, obrazovanje, iskustvo u poljoprivredi).

U nedostatnoj domaćoj literaturi postoje određena relevantna istraživanja o klimatskim promjenama. Tako se Ančić i sur. (2016) usredotočuju na opće stavove hrvatskih građana prema klimatskim promjenama, analizirajući njihovu procjenu ozbiljnosti, prijetnje okolišu i društveno-kulturne čimbenike koji utječu na proekološko ponašanje. Jug (2016) govori o širim implikacijama klimatskih promjena na poljoprivredu, naglašavajući potrebu za interdisciplinarnim pristupima i integracijom održivih praksi u sustave proizvodnje hrane. Branković i sur. (2009) naglašavaju značajnu ranjivost poljoprivrednog sektora s obzirom na klimatske varijabilnosti, uključujući štetu od suša, poplava i tuče. Naglašena je i potreba za mjerama prilagodbe i angažmanom javnosti u rješavanju utjecaja klimatskih promjena, sugerirajući da je razumijevanje percepcije poljoprivrednika ključno za razvoj učinkovitih strategija za ublažavanje tih izazova u Hrvatskoj. Žutinić i Zrakić Sušac (2021) u svom radu analiziraju percepciju klimatskih promjena od strane poljoprivrednih savjetnika. Istraživanje je pokazalo da su potonji relativno svjesni antropogenih uzroka i posljedica klimatskih promjena. Većina ispitanika smatra da poljoprivrednicima nedostaje potrebno znanje za učinkovito rješavanje rizika od klimatskih promjena. Savjetnici su izrazili snažnu suglasnost s potrebom političke i građanske odgovornosti u ublažavanju klimatskih promjena. Iz

prethodno navedenog potrebno je provesti i empirijsko istraživanje o percepciji vlastite spremnosti za klimatske promjene na populaciji poljoprivrednih proizvođača u Hrvatskoj.
Cilj rada je utvrditi kako poljoprivrednici doživljavaju utjecaj klimatskih promjena te utvrditi koje metode trenutno koriste za prilagodbu. Podcilj rada je utvrditi postoji li statistički značajna razlika kod istraživanih stavova i mišljenja obzirom na socio-demografske podatke.
Dobiveni rezultati pomoći će u smanjenju rizika od klimatskih promjena, poboljšanju sposobnosti poljoprivrednika za prilagodbu i izradi planova za buduće prilagodbe.

MATERIJAL I METODE RADA

Sudionici i prikupljanje podataka
Analiza se temelji na podacima prikupljenim u okviru šireg istraživanja stavova i mišljenja poljoprivrednika o klimatskim promjenama (Gregorić, 2024). Istraživanje je provedeno na neprobabilističkom prigodnom uzorku poljoprivrednika – vlasnika obiteljskih poljoprivrednih gospodarstava (OPG) u Republici Hrvatskoj. Podaci su prikupljeni od travnja do lipnja 2024. godine kombiniranjem usmene (*face-to-face)* i online ankete s primjenom istovjetnog upitnika.
Izravno anketiranje provedeno je na 25. Proljetnom međunarodnom bjelovarskom sajmu u trajanju tri dana sa svrhom testiranja razumljivosti pitanja, s obuhvatom od 15 ispitanika koji su pristali na sudjelovanje. Potom je anketni upitnik postavljen online pomoću alatke Google obrazac (*Google form*s). Poziv i poveznica za sudjelovanje u internetskoj anketi upućen je nositeljima OPGa putem društvenih mreža, online servisa za kupnju i prodaju domaćih proizvoda OPG-a u Hrvatskoj (OPGBurza.com, Lokalitet.hr, Burzahrane.hr i dr.) te telefonskim pozivima poljoprivrednicima s kojima je stupljeno u kontakt putem digitalne platforme OPG Hrvatska. Internetskoj anketi pristupilo je 89 poljoprivrednika od koji je 85 u potpunosti ispunilo anketu. Ostvareni uzorak sastojao od 100 sudionika, odnosno 65 muškaraca i 35 žena. Prosječna dob anketiranih poljoprivrednika iznosi 40,79 (~41) godina, najmlađi ispitanik ima 19 godina, a najstariji 67 godina. Ostali podaci o uzorku su prikazani u Tablici 1

Tablica 1. Sociodemografska obilježja uzorka

Varijabla		%
Spol	M	65
	Ž	35
Dob	19 do 40	56
	41 i više	44

Školska naobrazba	Osnovna škola	1
	Srednja škola	47
	Preddiplomski studij, viša škola	24
	Diplomski studij	27
	Poslijediplomski studij (doktorat i sl.)	1
Razina poljoprivredne naobrazbe	Tečaj iz poljoprivrede	44
	Završena srednja poljoprivredna škola	9
	Završena viša poljoprivredna škola, poljoprivredni fakultet	13
	Bez obrazovanja, samo iskustvo	34
Poljoprivreda u prihodima kućanstva	Jedini izvor prihoda	23
	Više od 50 %	41
	Manje od 50 %	36

Mjerni instrumenti

Anketni upitnik sadrži ukupno 13 pitanja podijeljenih u tri tematske cjeline: (i) percepcija klimatskih promjena, rizici i načini prilagodbe klimatskim promjenama, (ii) izvori informiranja o klimatskim promjenama i (iii) socio-demografska pitanja.

Mjerni instrument za *Opće stavove o klimatskim promjenama* sastojao se 16 izjava (vidi Žutinić i Zrakić, 2021) na koje su sudionici slaganje sa svakom izjavom procjenjivali na ljestvici Likertova tipa od pet stupnjeva, na kojoj 1 znači Uopće se ne slažem, a 5 znači Potpuno se slažem. U ovome članku prikazuju se odabranih šest tvrdnji koje su postigle prosječno najviši stupanja slaganja među poljoprivrednicima.

Izjave o *rizicima* (7 čestica) i *načini prilagodbe klimatskim promjenama* (9 čestica) kreirani su na temelju postojeće literature. Svoj stupanj slaganja/neslaganja s navedenim rizicima klimatskih promjena ispitanici su također, izražavali na ljestvici Likertova tipa od 5 stupnjeva. Pouzdanost korištene S*kale rizika* iznosila je Cronbach-α = 0,779.

Načini prilagodbe klimatskim promjenama mjereni su stupnjem implementacije od 1 do 3, gdje 1 označava nije primijenjeno; 2 djelomično, a 3 u potpunosti primijenjeno. Primjenom Cronbachovog koeficijenta utvrđeno je da ova skala pokazuje prihvatljivu pouzdanost, α = 0,701.

Obrada podataka

Anketni podaci podvrgnuti su deskriptivnoj analizi za izračun frekvencija, postotaka, srednje vrijednosti, standardne devijacije itd. Postojanje statistički značajnih razlika u percepciji, rizicima i prilagodbe klimatskim promjenama s obzirom na socio-demografska obilježja ispitanika provjereno je primjenom Pearsonovog hi-kvadrat testa (χ^2) uz toleranciju moguće pogreške od 5 % ($p < 0,05$).

REZULTATI RADA I DISKUSIJA

Neka prethodna istraživanja

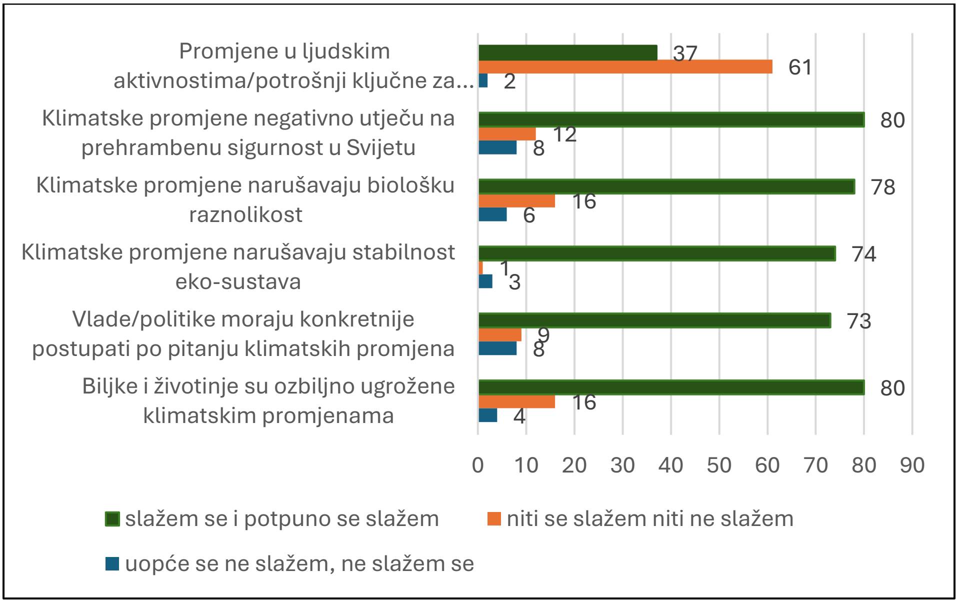
Stavovi i mišljenja poljoprivrednika o utjecaju klimatskih promjena izravno utječu na njihovu prilagodbu uvjetima okoliša, izboru usjeva i termina sjetve, metodama navodnjavanja i strategiju upravljanja gospodarstvom (Thav i sur., 2023). Istraživanje Yanga i sur. (2024) je pokazalo da percepcija poljoprivrednika o ekstremnim vremenskim prilikama ima značajno pozitivan utjecaj na njihovo adaptivno ponašanje u odnosu na klimatske promjene. Utvrđeno je da 86,68% (uzorak od 443 poljoprivrednika) poljoprivrednika usvaja prilagođena ponašanja kako bi smanjili rizike suočavanja s klimatskim promjenama, i da su razumijevanje i prepoznavanje klimatskih promjena ključni za poljoprivrednike kako bi poduzeli odgovarajuće mjere.

Istraživanja (Yang i sur., 2024; Kainyande, 2024; Raman i sur., 2024; Yadav i sur., 2023) pokazuju da je velika većina poljoprivrednika svjesna klimatskih promjena kao kritičnog i hitnog problema, što ih motivira da prilagode svoje poljoprivredne prakse - poput mijenjanja termina sadnje/sjetve i eksperimentiranja s različitim vrstama usjeva - na temelju rizika koje smatraju povezanim s tim promjenama u okolišu. Međutim, odnos koji postoji između percepcije poljoprivrednika i njihovih prilagodbi u ponašanju (ili odgovora/strategija prilagodbe) nije jednostavan, budući da ga isto tako oblikuju i brojni socio-demografski čimbenici, uključujući ali ne ograničavajući se samo na dob, obrazovanje i prethodna poljoprivredna iskustva.

Percepcija upravljanja rizikom od klimatskih promjena oblikuje odgovor poljoprivrednika odnosno strategiju prilagodbe. Razina sklonosti riziku značajno utječe na proces donošenja odluka (npr. odabir usjeva i navodnjavanje). Istodobno reakcije poljoprivrednika na klimatske promjene mogu biti vrlo heterogene, pod utjecajem čimbenika kao što su individualna averzija prema riziku, zadovoljstvo, nesigurnost, interakcija i usporedba s drugim poljoprivrednicima te prethodna iskustva (Gazzoti i sur., 2024).

Percepcija o klimatskim promjenama

Klimatske promjene su jedan od najznačajnijih izazova s kojima se suvremeno društvo suočava, a njihovi učinci na biljke i životinje su vrlo izraženi. Prema provedenom istraživanju (Graf 1.), prosječna ocjena percepcije ugroženosti biljaka i životinja zbog klimatskih promjena iznosi 4,74, što ukazuje na široko raširenu zabrinutost među ispitanicima. Osim toga, rezultati pokazuju da se većina ispitanika slaže s tvrdnjom da vlade i politike moraju konkretnije pristupiti problemu klimatskih promjena, s prosječnom ocjenom od 4,36. Rezultati pokazuju kako klimatske promjene značajno narušavaju stabilnost ekosustava i biološku raznolikost (4,26). Pored toga, ispitanici percipiraju negativan utjecaj klimatskih promjena na globalnu prehrambenu sigurnost (4,16), što dodatno naglašava potrebu za hitnim društvenim i političkim djelovanjem. Iako se promjene u ljudskim aktivnostima i potrošnji smatraju ključnima za ublažavanje posljedica klimatskih promjena, njihova prosječna ocjena iznosi 3,47, što ukazuje da su poljoprivrednici nedovoljno svjesni važnosti ovog aspekta. Zanimljivo je istaknuti da je 61 % ispitanika neutralnog stupnja slaganja s izjavom da su promjene u ljudskim aktivnostima/potrošnji ključne za ublažavanje klimatskih promjena. Prema istraživanju Žutinić i Zrakić Sušac (2021) poljoprivredni savjetnici su u suglasju s tom izjavom na razini 62,1 %, a indiferentnih je 32 %, što upućuje na razlike u percepciji.



Graf 1. Stupanj suglasnosti s izjavama o klimatskim promjenama (%), N=100

Rezultati hi kvadrat testa (χ^2) jedinu statističku značajnost pokazali su kod spola kao nezavisne varijable pri formiranju suglasnosti za tvrdnju da vlade/ politike moraju konkretnije postupati po pitanju klimatskih promjena (χ^2=9,623; p=0,047).

Percepcija upravljanja rizicima

U kontekstu poljoprivredne proizvodnje, klimatske promjene prema anketiranima, uzrokuju značajne ekonomske gubitke (Tablica 2.), s ocjenom 4,22, te negativno utječu na poljoprivrednu proizvodnju u Hrvatskoj (4,14). Zabilježen je visok stupanj suglasnost o nužnosti uvođenja i primjene klimatski i okolišno korisnih poljoprivrednih praksi, što je ocijenjeno prosječnom ocjenom 4,03. Ipak, postoji zabrinutost da općenito poljoprivrednici možda nisu dovoljno svjesni rizika koje klimatske promjene predstavljaju za proizvodnju (3,46). Prema istraživanju Raman i sur. (2024) iskustva poljoprivrednika s vremenskim promjenama utječu na njihova stajališta o klimatskim promjenama. Iskusni poljoprivrednici primjećuju promjene u vremenskim obrascima i sezonama rasta, dok oni s manje iskustva nisu toliko svjesni tih promjena. S vremenom, iskusni poljoprivrednici postaju sve osjetljiviji na klimatske promjene.

Tablica 2. Stupanj suglasnosti s izjavama o upravljanju rizicima (N=100)

Izjave	**Stupanj slaganja* %**			**mean**	**St. dev.**
	1+2	3	4+5		
Klimatske promjene uzrokuju velike ekonomske gubitke u lokalnim poljoprivredama.	6	15	79	4,22	0,980

Klimatske promjene negativno utječu na poljoprivrednu proizvodnju u Hrvatskoj.	7	15	78	4,14	1,015
Nužno je uvođenje i primjena poljoprivrednih praksi korisnih za klimu i okoliš.	10	16	74	4,03	1,068
Poljoprivrednici trebaju utjecati na donošenje odluka koje se tiču klimatskih promjena.	10	15	75	3,99	1,124
Klimatske promjene pridonose smanjenju obradivog zemljišta u Svijetu.	21	17	62	3,65	1,250
Poljoprivreda ima pozitivnu ulogu u ublažavanju klimatskih promjena.	15	34	51	3,57	1,130
Poljoprivrednici, farmeri nisu dovoljno svjesni rizika klimatskih promjena na poljoprivrednu proizvodnju.	20	28	52	3,46	1,167

* 1=Uopće se ne slažem, 2=Ne slažem se, 3=Niti se slažem ni ne slažem, 4= Slažem se, 5=Potpuno se slažem

Rezultati hi kvadrat testa (χ^2) jedinu statističku značajnost pokazali su kod dobi ispitanika kao nezavisne varijable pri formiranju suglasnosti za tvrdnju da poljoprivrednici, farmeri nisu dovoljno svjesni rizika klimatskih promjena na poljoprivrednu proizvodnju (χ^2=17,205; p=0,025). Mlađi ispitanici se više slažu s tom izjavom.

Strategija prilagodbe ili odgovor na klimatske promjene

Zbog sve izraženijih klimatskih promjena i njihovog utjecaja na poljoprivrednu proizvodnju, prilagodba poljoprivrednih praksi postaje ključna za očuvanje održivosti i produktivnosti. Istraživanje različitih strategija prilagodbe, kao što su uvođenje zelene gnojidbe, uzgoj otpornijih kultura, te izgradnja sustava navodnjavanja, ukazuje na varijabilne razine implementacije među poljoprivrednicima (Tablica 3.). Prema odgovorima, većina ispitanika pokazuje određeni stupanj spremnosti za uvođenje poljoprivrednih praksi korisnih za klimu i okoliš. Najzastupljenije su prakse zelene gnojidbe, uvođenja novih kultura, sorti ili pasmina otpornijih na promjene klime i uvođenje navodnjavanja kao agrotehničke mjere. Prema istraživanju Thav i sur. (2023) poljoprivrednici koriste razne strategije za prilagodbu klimatskim promjenama, uključujući promjene datuma sadnje/berbe, sorte riže, ulaganja u navodnjavanje i promjene razine inputa. Najčešća strategija je promjena datuma sadnje/berbe, koju koristi 35% poljoprivrednika, što se smatra najefektivnijom mjerom. Oko 20% poljoprivrednika mijenja sorte riže, koristeći one otporne na sušu, poplave i štetočine, što je u skladu s ovim istraživanjem.

Tablica 3. Stupanj implementacije dobrih praksi za ublažavanje posljedica klimatskih promjena (%)

Načini prilagodbe	**Stupanj implementacije %**		
	U potpunosti	**Djelomično**	**Ne, nije primijenjeno**
Uvođenje zelene gnojidbe	31	48	21
Uvođenje uzgoja kultura, sorti ili pasmina otpornijih na klimatske promjene	21	58	21
Izgradnja sustava navodnjavanja poljoprivrednog zemljišta	29	31	40
Osiguranje proizvodnje od gubitaka uzrokovani sušama, poplavama	22	27	51
Prijelaz na ekološku proizvodnju	18	31	51
Uspostavljenje poljskih traka	18	27	55
Primjena antierozivnih mjera na zemljištu	9	36	55
Izgradnja drenažnog i odvodnog sustava na poljoprivrednom zemljištu	12	26	62
Postavljanje mreža protiv tuče, mraza	11	15	74

Rezultati hi kvadrat testa (χ^2) pokazali su da prihod od poljoprivrede kao nezavisna varijabla značajno utječe na razinu uvođenja zelene gnojidbe (χ^2=11,047; p=0,026) i na uvođenje uzgoja kultura, sorti ili pasmina otpornijih na klimatske promjene (χ^2=13,314; p=0,010). Također test je pokazao da razina naobrazbe značajno utječe na uvođenje prilagodbe kroz izgradnju sustava navodnjavanja poljoprivrednog zemljišta (χ^2=7,213; p=0,027).

Navedeni načini prilagodbe zahtijevaju viša ulaganja u proizvodnji, ali i pretpostavljaju višu razinu educiranosti i informiranosti proizvođača. Neka ranije istraživanja (Thav *i sur.*, 2024) navode da iskustva poljoprivrednika s vremenskim promjenama utječu na njihova stajališta o klimatskim promjenama.

ZAKLJUČAK

Istraživanje percepcije hrvatskih poljoprivrednika o klimatskim promjenama i njihovim strategijama prilagodbe daje važne uvide u način na koji se poljoprivrednici suočavaju s izazovima koje donosi promjena klime. Rezultati pokazuju da većina poljoprivrednika prepoznaje ozbiljnost klimatskih promjena i njihovu prijetnju ekosustavima, biološkoj raznolikosti te globalnoj prehrambenoj sigurnosti. Najvažniji nalazi istraživanja su:

- 61 % ispitanika niti se slaže niti ne slaže s izjavom da su promjene u ljudskim aktivnostima/potrošnji ključne za ublažavanje klimatskih promjena;
- spol ispitanika ima statistički značajan utjecaj na suglasnost za tvrdnju da vlade/politike moraju konkretnije postupati po pitanju klimatskih promjena;

- mlađi ispitanici se u većoj mjeri suglasni da poljoprivrednici, farmeri nisu dovoljno svjesni rizika klimatskih promjena na poljoprivrednu proizvodnju;
- najvažnije prakse za ublažavanje posljedica klimatskih promjena prema istraživanju su uvođenje zelene gnojidbe, uvođenje novih kultura/sorti prilagođenih klimatskim promjenama i izgradnja sustava navodnjavanja;
- prihod od poljoprivredne proizvodnje i razina obrazovanja važan su faktor pri implementaciji praksi za ublažavanje posljedica od klimatskih promjena.

Ovo istraživanje ima više karakter eksplorativnog istraživanja te je proces definiranja uzorka manje pouzdan za poopćavanje rezultata na cijelu populaciju poljoprivrednika (jedinice uzorka uključene su prema kriteriju dostupnosti i pogodnosti). Također, uzorkom je obuhvaćen vitalniji dio populacije poljoprivrednika što je predvidljiv pokazatelj obzirom i na način prikupljanja podataka. Preporuka je raditi daljnja istraživanja na reprezentativnim uzorcima.

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HOW CROATIAN FARMERS PERCEIVE AND ADAPT TO CLIMATE CHANGE- PRELIMINARY RESEARCH RESULTS

Abstract

Climate change presents a significant challenge to the agricultural sector, necessitating a thorough understanding of farmers' perceptions and adaptation strategies for effective public policy development. A survey of 100 family farms in Croatia demonstrated a high level of awareness among farmers regarding the threats posed by climate change to ecosystems, biodiversity, and food security. They emphasized the need for more concrete government action and acknowledged substantial economic losses.
While there is a consensus on the importance of climate-friendly agricultural practices, concerns persist regarding insufficient awareness of climate change risks. Common adaptation strategies employed by farmers include green manuring, the cultivation of resilient crops, and the implementation of irrigation systems. Income and education levels were shown to influence the adoption of these measures, highlighting the significant role of socioeconomic factors in adaptation.
Farmers recognize the direct impact of climate change on their livelihoods and the urgency for action. The survey underscored the necessity for enhanced farmer education on climate change risks and the promotion of climate-friendly practices. Furthermore, the government must implement more decisive policies to support farmers in their adaptation efforts. To ensure the generalizability of these findings, further research using a more representative sample is recommended. This will facilitate a more comprehensive understanding of the challenges faced by Croatian farmers and contribute to the development of effective climate change adaptation strategies.

Keywords: *climate change, farmers, perception, adaptation, strategies*

THE COST PRICE OF GROWING LAVENDER (*LAVANDULA OFFICINALIS*) AND THE ECONOMIC EFFICIENCY OF ITS PROCESSING IN THE TERRITORY OF THE FEDERATION OF BOSNIA AND HERZEGOVINA*

Šejla Burazerović[1], Emir Bećirović[1]

Original scientific paper

Abstract

Lavender is one of the Lamiaceae family's most famous ornamental, aromatic, and medicinal plants. It is widely used in the cosmetic, pharmaceutical and food industries and is an excellent honey plant. The plantation cultivation of lavender in BiH is still not as developed as in other countries. Still, an increasing number of farms and specialized farms recognize this culture's potential. Given the need for more research on the economic results of growing lavender in Bosnia and Herzegovina, this research aims to analyze the cost of growing fresh lavender and the economic efficiency of its processing. Primary data was collected with the help of surveys conducted on four farms, focusing on the necessary investments and operational costs of growing lavender and the income from the sale of dried flowers. The results showed that the cost of growing 1 kg of fresh lavender flowers ranges from 0.71 BAM to 1.33 BAM, which indicates significant differences between the observed farms. Lavender processing also showed different levels of economy among farms. According to this research, cultivating lavender in Bosnia and Herzegovina is an economically profitable option; however, it is necessary to research a more significant number of farms. Also, due to different applied agricultural techniques in the cultivation of lavender, economic results differ significantly from farm to farm, so one of the research recommendations is additional education of producers in this area to increase the economic efficiency of these farms with the improvement of production technology.

Keywords: *economic efficiency, cost price, lavender, Federation of Bosnia and Herzegovina*

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INTRODUCTION

Lavender is an aromatic, ornamental and medicinal plant known for its healing properties and wide application in the cosmetic, pharmaceutical, food and decorative industries (Gadzo, 2017). It grows at an altitude of up to 1,700 m above sea level, and its habitat is bogs, rock gardens and pastures (Jurkic, 2014). This plant is characterized by exceptional resistance, capable of withstanding temperatures as low as -20oC (Mihovilovic, 2005). For successful production, knowing and applying correct agrotechnical methods is essential. As a perennial plant, it can last 15 to 20 years with proper care, ensuring a high yield and quality flowering (Grbesa, 2016). It requires sunny and warm conditions for optimal growth (Mihovilovic, 2005). It is traditionally grown in areas with a Mediterranean climate, but due to its resistance to low temperatures, it is also grown in continental and temperate-continental climates. There are more than 40 species of this plant; the most widely cultivated are Lavandula officinalis, Lavandula dentata, Lavandula stoechas and Lavandula x intermedi (Parida, 2022). A strong, distinctive smell from the accumulated essential oil characterizes all types of lavender.

Lavender is mainly processed in the dryer, where dried lavender is obtained, and through the distillery, where the essential oil is obtained through the distillation process, and the side product hydrolat is created. For 1 kg of dried lavender, 8-10 kg of fresh lavender is used (Siljes *et al*., 1992.). Various factors will influence the quantity and quality of the final dried product during the drying process. Drying parameters depend on environmental conditions and lavender varieties, which influence the final yield of dried lavender (Sałata, 2020). Different studies have shown that drying methods contribute considerably to moisture diffusion and energy consumption during the drying process. Similar results were found by Masztalerz *et al.* (2021), who conducted a study on combined drying methods, which resulted in a shortening of drying time and improved the quality of dried lavender. It has also been emphasized that drying techniques such as heat pump drying can retain the phytochemical composition of lavender more than traditional hot-air drying methods (Falla *et al*., 2022)

Essential oil is obtained by distillation of lavender flowers. Natural lavender contains 0.5 to 1.5% essential oil (Bozanov, 2018). Lavender essential oil is mainly used in the cosmetics, fragrance and perfumery industries. Nevertheless, there is an increasing interest in its use in aromatherapy (Singh *et al*., 2007). The essential oils extracted from dried lavender are highly valued in various industries, including food, cosmetics, and aromatherapy, further emphasizing the importance of effective drying methods to maintain quality (Łyczko *et al*., 2019).

The world`s largest lavender producers include the USA, Australia, South Africa, Algeria and Argentina, while in Europe, countries such as Bulgaria, England, France, Italy, Spain, Hungary, and the former Yugoslavia stand out (Gadzo, 2017). Apart from direct income for the farms that produce it, lavender can also contribute to the tourism segment of a country's economy, which speaks of its potential for developing agritourism in Bosnia and Herzegovina. Lavender cultivation is environmentally friendly for several reasons: no pesticides are used, and small amounts of water and mineral fertilizers are used, which do not create large amounts of waste (Zutic, 2014). Growing lavender is increasingly popular in Bosnia and Herzegovina thanks to favourable climatic conditions, and specialized farms recognize its potential. Although lavender production in Bosnia and Herzegovina is still in its early stages, the global demand for natural and aromatic herbs is growing, which opens opportunities for further development of this crop. Studies on lavender production or its economic impact in Bosnia and Herzegovina are minimal, so this research aims to analyse the cost of growing fresh lavender and the economic efficiency of its processing.

MATERIALS AND METHODS

The primary source of data is surveys conducted to collect information on the costs, yields, and incomes of four farms that grow lavender (Sarajevo, Visoko, Mostar and Stolac). Farm sizes and farm locations are Farm 1 (F1) with an area of 3.0 dunums in Visoko, Farm 2 (F2) with an area of 1.2 dunums in Mostar, Farm 3 (F3) with an area of 35.0 dunums in Stolac and Farm 4 (F4) with an area of 2.5 dunums in Sarajevo. Data was collected through personal interviews with farmers, during which information was collected on the costs of inputs such as seedlings, fertilizers, pesticides, labour, and others. Furthermore, data were collected on lavender yields, sales prices, and challenges and problems in production. After collecting and entering the data into Excel 365, a detailed economic analysis was performed to obtain basic information about these units' financial success. The study included assessing the necessary investments per dunum of lavender, the achieved average yields, and costs, which enabled the calculation of the cost price of fresh lavender. Special attention is paid to calculating the costs of drying lavender, including the costs of equipment for a dryer with a capacity of 300 kg, energy, and labour costs. A partial market analysis was carried out, which included the main sales channels of these products and their prices on the market. With this information, it was possible to obtain the cost price for growing lavender, as well as the expected profit for its drying.

The cost price for fresh Lavender is calculated according to the formula (Mulic, 1983):

Cost price = Total cost/Yields

Profit for drying lavender was calculated according to standard formula:

Profit = Revenues – Total cost

Economic efficiency for draying lavender is calculated based on next formula (Falan and Mujcinovic, 2022):

Economic Efficiency = Revenues/Total costs

RESULTS AND DISCUSSION

The presented data sequentially provides an overview of the average costs of cultivating lavender on an area of 1,000 m 2 across the analyzed farms. Further analyses summarize the costs and cost of lavender cultivation for the individual farm's total area. The last group of information offers a financial analysis of the costs involved in drying lavender using dryers with a 300 kg capacity.

Table 1. The average costs of cultivation lavender on the area of 1,000 m^2 of analyzed farms

(in BAM)

Description	**Unit**	**Amount**	**Price**	**Total**
Yields				
Lavender (flower)	kg	1,218	-	-
Fertilizer	kg	25	1.12	28
Pesticide	kg	0	-	0
Labour	day	10	65	650
Mechanization	day	3	100	300
Packaging				56
Depreciation				183
Other costs				90
TOTAL COST				**1,307**
Cost price				**1.07**

Table 1 comprehensively summarizes the average costs of cultivating lavender on 1,000 m^2 at observing farms. Here, we can see a detailed average calculation, specifically for the four observed cases, where the consumption of individual material inputs is presented along with their costs. The total costs of fertilizers, pesticides, labour, mechanization, packaging, and depreciation amounted to 1,307 BAM per 1,000 m^2. In the cost structure, the most significant are those associated with wages due to using manual labour, which accounts for nearly 50% (650 BAM) of the total annual costs. Mechanization also incurs significant expenses, making up slightly less than 25% (300 BAM) of the total expenses for maintaining lavender plantations. It is also important to emphasize the depreciation costs, which amount to 183 BAM. These are exclusively related to the depreciation of the lavender plantation and were calculated based on preliminary analyses, which considered the total costs for establishing such a plantation.

With average yields of 1.218 kg per dunum and the costs shown, 1 kg of fresh lavender cost was 1.07 BAM.
Similarly, detailed calculations were made for each farm, as shown in Table 2.

Table 2. Costs and cost of lavender cultivation on the total area for the analyzed farms **(in BAM)**

Description	**F1***	**F2***	**F3***	**F4***
Total Yields (kg)	3,000	2,500	43,750	1,560
Area of farm (1000m^2)	3	1	35	2.5
Yields per 1 dunum (kg)	1,000	2,084	1,250	624
Fertilizers	1,197	0	0	0
Pesticide	0	0	0	0
Labour	1,200	1,120	31,500	750
Mechanization	600	400	10,500	500
Packaging	167	0	2,188	0
Depreciation	600	168	6,195	530
Other costs	240	150	1,000	200
TOTAL COST	**4,004**	**1,838**	**51,383**	**1,980**
Cost per dunum	**1,335**	**1,838**	**1,468**	**566**
Cost price	**1,33**	**0,74**	**1,17**	**1,27**

*F1, F2, F3 and F4 are the labels for the four observed farms (Farm 1, Farm 2, Farm 3, Farm 4)

Table 2 shows that farm size significantly affects total costs, labour and mechanization use. Farm F3 has the highest yield at 43,750 kg, while F4 has the lowest yield at 1,560 kg. Yields per 1 dunum show a significant variation, with F2 achieving the highest yield of 2,084 kg, while F4 produces only 624 kg. Total costs are highest for the F3 farm, which is 51,383 BAM, corresponding to its larger area and higher yields. Farm F2 has the lowest total cost with an amount of 1,838 BAM, reflecting its small size and lack of additional inputs like fertilizer and packaging. The cost per kilogram is lowest for F2 (0,74 BAM), indicating more cost-efficient production, while F1 and F4 have relatively higher cost prices (1,33 BAM and 1,27 BAM). Farm F3 has a moderate cost of 1,17 BAM.
Such significant differences in the cost price of fresh lavender flowers are primarily due to the different technologies applied in lavender cultivation and different production intensities. These differences are most evident in the planting density of individual lavender plantations but also in the agro-techniques used throughout the year, including

the application of organic fertilizers and other technical practices such as timely soil treatment and fertilization.
Table 2 shows the differences in cultivation and maintenance technologies for lavender plantations when observing the various costs per dunum across the studied farms.
What is particularly interesting is that the farm with the highest costs per dunum in production achieves the lowest cost price for cultivated lavender. Although the highest costs per dunum amounted to 1,838 BAM, the yield was 2,084 kg of fresh lavender flowers, resulting in a cost price of only 0.74 BAM per kg. The farm with the lowest costs per dunum, amounting to 566 BAM, achieves a high-cost price (1.27 BAM/kg) for lavender produced.
This shows how the application of higher intensive agrotechnical measures, even with a higher cost per unit of production (1.000 m2), benefits in achieving better economic results.

Table 3. Financial analysis of drying Lavender in dryers with 300 kg capacity

(in BAM)

Description	**F1**	**F2**	**F3**	**F4**
Dry lavender (kg)	334	278	5250	173
Price	13	13	13	13
Total Revenues	4,336	3,614	68,250	2,253
Lavender	4,004	1,838	51,383	1,980
Energy costs	280	252	4,424	146
Labor costs	200	280	3,150	450
Maintenance costs	120	120	120	120
Packaging	17	14	263	9
Depreciation	460	460	460	460
Other costs	180	110	950	160
TOTAL COSTS	5,261	3,074	60,750	3,325
PROFIT	**-925**	**540**	**7,500**	**-1,072**
Cost price	**15.75**	**11.06**	**11.57**	**19.22**
Economic Efficiency	**0.82**	**1.18**	**1.12**	**0.68**

Table 3 provides a financial breakdown of the revenue, costs, and profit from drying lavender across four farms: F1, F2, F2, and F4. It includes total revenues, costs, and profits, offering a comparison of each farm's financial efficiency. Those are hypothetical results that are not found on all observed farms. We wanted to check how investments in a drawer with 300 kg capacity could impact the economic results of those farms. Findings can be seen in Table 3.
Farm F3 produces the highest amount of dried lavender, 5,250 kg, while F4 produces the lowest, 173 kg. The price of dried lavender per kilogram is the same for each farm, which was the case on the market in this period. Revenues are highest for F3, with 68,250 BAM due to its large production, while F4 generates the lowest income of 2,253

BAM. The costs between farms are different due to various factors, one of the most important of which is the amount of lavender production on the farm. Most of the costs go to lavender production, and other costs are included, such as energy and labour costs, maintenance costs, packaging, depreciation and other costs. The total costs are the highest for farm F3, which amounts to 60,750 BAM, followed by F1 with 5,261 BAM, F4 with 3,325 BAM, and F2 with 3,074 BAM. Farms that have positive profits are F2 and F3, but F2 generates a more balanced revenue-to-cost ratio. F1 and F3 both experience losses, with F1 having a negative profit of -925 BAM and F4 losing -1,072 BAM. The price per kilogram of dried lavender varies significantly, with F4 having the highest cost price of 19,22 BAM, making it less efficient than F2, which has the lowest cost price of 11,06 BAM. Farms F1 and F3 have intermediate cost prices. Efficiency is highest for F2 with 1,18, indicating that it generates the most revenue relative to its costs. F3 with efficiency 1,12 is also relatively efficient. Farms F1 and F4 have an efficiency of less than 1, showing that their costs are higher than revenue, so these two farms are not economically efficient in producing dried lavender.

Now, it is possible to see how the lower cost of fresh lavender impacts the financial results of dried lavender. F2, although the smallest in terms of area, achieves by far the lowest cost of lavender cultivation, so the investment in a dryer is worthwhile, regardless of the small volume of production. Also, the largest of the observed F3, shows positive results when investing in a small capacity dryer of 300 kg. For this size of farm, it would be interesting to make a financial analysis of investments in dryers of larger capacity. This would reduce drying time and the use of labour for this process. For F1 and F2, the cost of dried lavender, under this condition, is too high, and the selling price is lower than the costs per kg of dry lavender produced.

CONCLUSION

The economic outcomes of lavender cultivation vary considerably across farms due to differences in agricultural practices, farm sizes and production techniques. This variability highlights the importance of further education and training for producers to enhance the economic efficiency of lavender farming; by improving production technology and applying optimal agrotechnical practices, farmers can significantly increase their profitability. Based on this research, lavender cultivation in Bosnia and Herzegovina can be economically viable, provided that adequate farming techniques are employed. However, for a more comprehensive understanding of the economic potential of this crop, it is essential to gather data from a larger sample of farms. Expanding the scope of the research would allow for more precise conclusions and better-informed recommendations for the industry. Additionally, when considering potential investments in small-capacity dryers on these farms, it becomes evident that, with appropriate and timely agrotechnical practices, positive results can be achieved even on very small farms. Agro techniques need to be well implemented, and yields need to be higher per production unit. Future research should be implemented for larger farms about investments in higher-capacity dryers, which would increase depreciation costs, but potentially, other running costs could be lower.

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CIJENA KOŠTANJA UZGOJA LAVANDE (*LAVANDULA OFFICINALIS*) I EKONOMSKA UČINKOVITOST NJEZINE PRERADE NA PODRUČJU FEDERACIJE BOSNE I HERCEGOVINE

Abstract

Lavanda je jedna od najpoznatijih ukrasnih, aromatičnih i ljekovitih biljaka porodice Lamiaceae. Široko se koristi u kozmetičkoj, farmaceutskoj i prehrambenoj industriji i odlična je medonosna biljka. Plantažni uzgoj lavande u BiH još uvijek nije toliko razvijen kao u drugim zemljama. Ipak, sve veći broj farmi i specijaliziranih farmi prepoznaje potencijal ove kulture. S obzirom na potrebu za dodatnim istraživanjima ekonomskih rezultata uzgoja lavande u Bosni i Hercegovini, ovo istraživanje ima za cilj analizirati cijenu uzgoja svježe lavande i ekonomsku efikasnost njene prerade. Primarni podaci prikupljeni su uz pomoć anketa provedenih na četiri farme, s fokusom na potrebna ulaganja i operativne troškove uzgoja lavande i prihode od prodaje sušenog cvijeća. Rezultati su pokazali da se cijena uzgoja 1 kg svježeg cvijeća lavande kreće od

0,71 KM do 1,33 KM, što ukazuje na značajne razlike između posmatranih farmi. Prerada lavande je takođe pokazala različite nivoe ekonomičnosti među farmama. Prema ovom istraživanju, uzgoj lavande u Bosni i Hercegovini je ekonomski isplativa opcija; međutim, potrebno je istražiti veći broj farmi. Također, zbog različitih primijenjenih poljoprivrednih tehnika u uzgoju lavande, ekonomski rezultati se značajno razlikuju od farme do farme, pa je jedna od preporuka istraživanja dodatna edukacija proizvođača u ovoj oblasti za povećanje ekonomske efikasnosti ovih farmi uz unapređenje proizvode tehnologije.

Keywords: *economic efficiency, cost price, lavender, Federation of Bosnia and Herzegovina*

THE ECONOMIC EFFICIENCY OF GEOTHERMAL HEATING SYSTEM IN SMALL-SCALE GREENHOUSE OPERATIONS IN BOSNIA AND HERZEGOVINA*

Emir Bećirović[1], Sabrija Čadro[1], Merima Makaš[1], Ćerima Zahirović Sinanović[1], Zuhdija Omerović[1]

Original scientific paper

Abstract

Greenhouse vegetable production offers many advantages over traditional open-field cultivation methods. These benefits include regulating and controlling growing conditions, leading to higher crop yields, year-round production, and increased resource allocation efficiency. Many studies demonstrate that heating systems improve production in greenhouses, leading to increased yields and improved economic profitability. However, similar research is lacking in Bosnia and Herzegovina, particularly in regions with a continental climate. Therefore, the main goal of this study was to evaluate the economic efficiency of vegetable production (lettuce, spring onion, tomato, and spinach) in a greenhouse equipped with an additional heating system compared to one without such a system. The experiment was conducted in two 100 m^2 greenhouses using identical cultivation technology. The results showed that the temperature in the two separate greenhouses was statistically significantly different in winter; however, the observed yields did not show a statistically significant difference. Unexpectedly, the economic results were contrary to expectations, with lower returns in the greenhouse with heating. The main reason is the higher costs associated with deprecation (1,238 BAM) and the geothermal pump's electricity consumption (700 BAM). In conclusion, through this research, it was found that the tested vegetable production using a geothermal heat pump is not economically justified. It is recommended to consider the use of such equipment under different conditions, such as larger greenhouse areas with improved insulation (double-layered plastic). Furthermore, it is recommended that similar experiments be conducted at this location to confirm or challenge the results obtained in this study.

Keywords: *economic efficiency, greenhouses, Bosnia and Herzegovina, geothermal heating system, continental climate*

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INTRODUCTION

Vegetable production in greenhouses offers several advantages over traditional outdoor production. These advantages include the ability to regulate growing conditions, which contributes to higher crop yields, overall production, and improved resource efficiency (Laktionov *et al*., 2020; Odhiambo *et al*., 2021; Zhao *et al*., 2022; Zhou *et al*., 2020). With the increase in the world population and the decrease in arable land, controlling conditions in greenhouses is essential to ensure safe food production (Kuswardhani *et al*., 2014). Greenhouses enable the application of intensive agricultural practices, such as irrigation and tillage operations, resulting in higher yields and more efficient production processes (Çanakcı & Akinci, 2006). Studies have also found that applying agrochemicals, both chemical and organic fertilisers, in greenhouse vegetable systems is higher than in open-field systems, contributing to higher yields in greenhouses (Yuan & Zhang, 2021). Furthermore, research indicates that greenhouse cultivation generally promotes better growth parameters, such as height and width extension, leaf number, leaf area, average leaf size, and total leaf dry mass compared to outdoor conditions (Zhang *et al*., 2021). Also, it encourages increased vegetable yields and promotes carbon accumulation in the soil, highlighting the benefits of vegetable production within greenhouses (Laktionov *et al*., 2018). Furthermore, the impact of heating on greenhouse yields has been a subject of interest. Samaranayake *et al.* (2020) analysed the seasonal effects on greenhouse energy consumption during crop production, emphasising the importance of environmental control in achieving higher yields. The profitability of greenhouse production can be related to the intensity of energy invested in production and the quality and volume of output products (Tong, 2024). Economic analyses show that greenhouse vegetable production is more profitable than outdoor production (Hao *et al*., 2009; Yajie, 2023).

Additionally, greenhouse heating systems can create optimal conditions for vegetable crops, increasing yields. Efficient greenhouse heating is essential for minimising production costs and creating optimal conditions for plant growth. Winter heating can account for a significant portion of greenhouse production costs, highlighting the need for sustainable heating solutions (Blanco *et al.*, 2013). Various heating systems have been studied to improve energy efficiency and reduce greenhouse production costs. Energy consumption for greenhouse heating can be substantial, indicating the importance of exploring efficient heating methods (Wang & Liang, 2006). Studies have also delved into optimising energy consumption and microclimate control in greenhouses. Optimised daily average temperatures in a greenhouse to minimise energy consumption, providing insights for decision-making in greenhouse heating (Shen *et al*., 2018).

Biomass heating systems in rural China demonstrate innovative approaches to meet heating and greenhouse demands sustainably (Huang *et al*., 2020). Bezari *et al.* (2020) focus on rock-bed heat storage systems to preserve thermal energy and respond to temperature variation in greenhouses, stating the importance of thermal storage during day and night-time for stable microclimates. Experimental studies of a geothermal heat pump's performance for greenhouse heating illustrated how low-enthalpy geothermal sources can be used to meet agricultural needs (Anifantis *et al*., 2016). Barbaresi *et al.* (2020) evaluated the efficiency of a geothermal heat pump system for greenhouse heating, underlining the importance of sustainable heating solutions.

In BiH, there is limited research on the profitability of greenhouse production (Becirovic *et al*., 2018; Čadro *et al.*, 2022)Given the higher yield potential per unit area and the increased safety of production, particularly in relation to climate change risks such as early spring heat waves followed by sudden temperature drops, this type of production could potentially offer solutions for many smallholder farmers in BiH.
However, studies that directly address the economic profitability of heating greenhouses for winter vegetable production remain scarce. This was the main reason for conducting this study at the Butmir experimental field, with the aim of evaluating the profitability of such systems under the continental climate conditions of BiH.

Ha1 – A statistically significant difference exists between the temperatures the heated and the control greenhouses achieved in the observed period.

Ha2 – There is a statistically significant difference in the realised yields in the heated and control greenhouses.

MATERIALS AND METHODS

In May 2020, two identical greenhouses, each covering an area of 100 m² (6.3 m x 16.0 m), were established side by side at the experimental site of the Faculty of Agriculture and Food Science at the University of Sarajevo, located in Butmir near Sarajevo.
Both greenhouses were constructed using materials and designs typical of those used by local farmers. However, one greenhouse was equipped with a geothermal heating system, while the other served as a control and was not heated. The installed geothermal heat pump (Ecoterm, Type 263, max power 3.2 kW) utilises soil heat collectors made of plastic pipes installed in trenches 120 cm deep and 60 cm wide. To meet the heating requirements for the 100 m² greenhouse, with a heating demand of 12 kW, three trenches, each 50 meters long, were dug for the earth collectors.
After analysing the heating capacity of the geothermal pump and the subsequent soil and air temperatures, the following operational protocol was implemented for the pump:
- The output temperature from the heat pump is set to 25°C.
- The geothermal pump operates simultaneously with an air fan.
- The pump is activated when the air temperature inside the greenhouse falls below 10°C.
- The pump is turned off when the temperature inside the greenhouse rises above 10°C.

The temperatures measured at 7:00, 14:00 and 21:00 hours are used to establish whether a statistically significant temperature difference was achieved with the help of heating in the two observed greenhouses.

The research was conducted on the following vegetable crops: lettuce, spring onion, tomato cv. Pink Rock, tomato cv. Mathias, and spinach during the period from November 2021 to April 2022. Data collection for the experiment was carried out directly on-site, where all data were entered into the CoboToolbox software. This software enabled data storage and partial analysis. The collected data include all material costs, such as planting material, fertiliser, protective equipment and other inputs, as well as detailed records of labor costs. These data made it possible to calculate the overall economic performance of the two greenhouses under study accurately. Product price trends during the season were determined based on monitoring average product prices during the research in several different markets, while selling prices for farmers were determined through a survey of five active producers of these products. Prices for consumed inputs were determined based on invoices submitted when purchasing inputs.
The gross margin (GM), a concept that covers variable costs, was used in the economic analysis. Specifically, the gross margin represents the difference between the achieved revenues and exclusively the variable costs (Kay *et al.*, 2015).

$$GM = Revenue – Variable cost$$

The concept of standard economic profit, which includes fixed costs, was also used. The following formula was used in this paper:

$$PROFIT = Gross margin – Depreciation$$

The study presents the expected revenues and costs for cultivating these crops on 25 and 100 square meters.

RESULTS AND DISCUSSION

Table 1 shows the vegetation period of all the crops grown during the experiment. The study demonstrates the impact of heated greenhouses on shortening the vegetation periods of various crops. Lettuce in heated greenhouses requires only 67 days, 49 days less than in unheated conditions. For spring onions, the vegetation period in the heated greenhouse was 30 days shorter than in the control. Spinach had a vegetation period of 52 days in heated greenhouses, significantly shorter by 50 to 130 days compared to unheated greenhouses in Bosnia and Herzegovina. For tomatoes, the difference in vegetation period between heated and unheated greenhouses was minimal, with only a seven-day reduction. Overall, heating significantly reduces vegetation, especially for lettuce, spring onions, and spinach. This shorter vegetative period in the heated greenhouse has allowed the growth of one more circle of production in the wintertime.

Table 1. Length of vegetation period of individual crops in control and heated greenhouses

Type of individual crops		Planting date	Harvesting date	Vegetation length in days
CONTROL	Lettuce	12.12.2020.	05.02.2021.	116
	Spring Onion	12.12.2020.	05.02.2021.	116
	Tomato Pink Rock	12.04.2021.	05.10.2021.	176
	Tomato Mathias	12.04.2021.	05.10.2021.	176
HEATED	Lettuce	12.10.2020.	18.12.2020.	67
	Spring Onion	12.10.2020.	05.01.2021.	85
	Spinach	05.01.2021.	25.03.2021.	52
	Tomato Pink Rock	05.04.2021.	05.10.2021.	169
	Tomato Mathias	05.04.2021.	05.10.2021.	169

Table 2 presents the average temperatures in two greenhouses, one heated and the other a control. Temperatures were measured at 7:00, 14:00 and 21:00 hours, and they were monitored from November to April. The table also includes the p-values of the test, showing where and during which period statistically significant differences in recorded temperatures occurred.

While significant differences were not observed at 14:00 h, except in January, statistically significant differences were achieved in temperatures measured at 7:00 and 21:00 h from November to February. No statistically significant differences in average temperatures were recorded in March and April.

Thus, we can state that the first hypothesis is partially confirmed, as statistically significant differences in recorded temperatures were observed in some cases within the two greenhouses. Overall, statistically significant differences were observed in 9 measurements out of 18 measurements. This allows us to conclude that the first hypothesis is partially confirmed.

Table 2. Average temperatures (in ºC) at 7:00, 14:00 and 21:00 h inside the control and heated greenhouse with statistical analysis of values using the t-test (α = 0.05) with statistical analysis of mean values (t-test) greenhouses

Month/ Hour	07:00		14:00		21:00		p value - t test		
	Contr	Heate	Contr	Heate	Contr	Heate	07:0	14:0	21:0
Novemb	-0.5	3.9	15.9	16.6	2.4	6.1	0.005	0.650	0.001
Decemb	1.7	6.1	10.9	13.3	3.0	7.2	0.000	0.113	0.000
January	-1.0	2.7	7.9	10.8	0.5	4.1	0.001	0.008	0.000
Februar	-0.6	2.1	17.6	18.8	1.2	4.0	0.020	0.578	0.018
March	1.9	3.0	17.1	16.8	3.0	3.9	0.259	0.854	0.259
April	1.2	4.1	19.6	19.1	4.3	5.3	0.295	0.893	0.502

*** Differences between temperatures are statistically significant**

The most significant differences in achieved temperatures are observed in December and January, with January standing out particularly, as statistically significant differences in recorded temperatures appeared in all three time measurements.
Expected investments in greenhouse production without a geothermal pump in BiH are 2,639 BAM. Considering the years of amortisation, the annual depreciation would be 176 BAM. However, if there is a geothermal pump on greenhouses, depreciation would be eight times higher, and the total investment would be 21,211 BAM. As a result, the annual amortisation would be 1,415 BAM. From this, we can see that it is necessary to achieve a higher gross margin of 1,239 BAM in the heated greenhouse compared to the unheated greenhouse for the same final financial results.

Table 3. Type of investments in greenhouse production with and without a heating system

(in BAM)

Investments	**Greenhouse**	
	Control	**Heated**
Greenhouse with standard equipment	2,639	2,639
Installation of hot air distribution pipes		94
Hot air distribution pipe		156
Installation of geothermal heating systems		18,330
Total amount of investments	**2,639**	**21,218**
Number of years of depreciation	15	15
Annual amount of depreciation	**176**	**1,415**

Table 4 presents the average yields of different crops in two separate greenhouses. At first glance, it can be concluded that, except for onions, no statistically significant yields were achieved in the heated greenhouse. For onions, yields in the heated greenhouse reached 155 kg per 100 m² compared to 87 kg per 100 m² in the unheated greenhouse.

Table 4. Achieved yields in control and heated greenhouse on the Butmir experimental field on the area of 100 m^2 for the different crops

(in kg)

Type of crops	**Greenhouses**	
	Control	**Heated**
Lettuce	337	326
Onion	87	155
Tomato - Mathias	1,956	1,332
Tomato - Pink Rock	1,732	1,540
Spinach	-	315

When observing individual crops, no significant difference in lettuce yields was recorded between the two greenhouses. Contrary to expectations, the tomato yields for the Matias and Pink Rock varieties were lower in the heated greenhouse compared to the control greenhouse. This certainly represents a surprising outcome in the study. However, the significant difference in achieved yields can be explained by the fact that tomatoes in the heated greenhouse were planted seven days earlier, and during the flowering period, one night saw exceptionally low temperatures. This damaged the first flowers in the heated greenhouse, as the heating system was unable to compensate for the temperature difference between the outside and inside, thus failing to provide optimal conditions for the plants during this critical period. For this reason, the first harvest of tomatoes from the heated greenhouse yielded lower results compared to the unheated greenhouse. Also, fruit deformity (catfacing) was noted in the heated greenhouse during the first harvesting period. It is known that tomatoes are highly sensitive to heat stress, especially in the flowering phenophase (Sikes and Coffey, 1976). Exposure to suboptimal temperatures during flowering leads to deformed fruits that have little to no market value. One such disorder is "catface", a deformity that appears on tomato fruits when exposed to temperatures below 10°C during critical growth periods (Masarirambi *et al*., 2009). Catfacing is the abnormal development of plant tissue affecting the ovary or female sex organ (pistilate), which results in the flower, followed by the fruit development to become malformed. The exact cause of catfacing on tomatoes is uncertain and could be caused by any number of factors, but it seems to centre around unfavourable growing conditions. Temperatures below 10°C for several successive days when plants are immature - about three weeks before blooming - appear to coincide with tomato catfacing fruit deformity. However, all these results should be taken with caution, and a more reliable conclusion could be made through two or three additional years of research in the same region, using the same greenhouses under similar conditions.

The most significant difference in the production plan between the two observed greenhouses is that an additional production cycle could be conducted in the heated greenhouse during the winter months. This study achieved this by growing an extra crop of spinach. These facts, combined with increased yields of other crops, were expected to compensate for the difference in depreciation costs and electricity consumption in the heated greenhouse. The following tables show how successfully this was achieved.

During the experiment's design, it was expected that the significantly higher depreciation for the heated greenhouse would be compensated by the higher yields achieved within it and by the anticipated higher prices for products that would reach the market earlier than those arriving later from the control greenhouse. However, during the study year, no significant difference was observed in the prices of products based on the timing of their market availability.

Table 5. Achieved gross margin and profit in the control and heated greenhouse on the Butmir experimental field on an area of 25 m^2 for the different crops

(in BAM)

Description	**Greenhouses**	
	Control	**Heated**
Gross margin of individual crops		
Lettuce	221	106
Onion	-55	-149
Tomato - Mathias	708	380
Tomato - Pink Rock	838	675
Spinach	-	493
Total gross margin	**2,230**	**2,119**
Depreciation	176	1,415
Profit	**2,054**	**704**

Thus, not only were the expected higher yields not achieved in the heated greenhouse, but higher prices for these products were also not realised. For this reason, even the total gross margins were lower in the heated greenhouse. This indicates that the costs in the heated greenhouse are higher, while the expected benefits were not achieved. For this, the financial results in controlled greenhouses in this experiment were 2,054 BAM, contrary to 704 BAM in heated greenhouses. So, in this case, with those yields and prices, it could be said that an option without heating is better for the farmer economically.

Further, it has been analysed what would happen if the farmers included in the production plan only production lines with the highest gross margin in both greenhouses from this experiment. This will enable us to make predictions about the highest expected gross margin possible in both greenhouses under the given circumstances.

The results of this experiment demonstrate that onion production will not be considered an economically viable production option in either greenhouse.

For this reason, the optimal production plan for the control greenhouse includes the most cost-effective crop during winter and another crop during spring/summer. Consequently, for control greenhouse the selected crops are letuce and the Pink Rock tomato variety, which proved slightly more profitable and cost-effective than the Matias variety. Using a geothermal pump during the winter allowed two production cycles in heated greenhouses. Spinach and lettuce were selected for consideration. Although spinach proved to have a higher gross margin for cultivation during the winter period, it was decided to grow two different crops during the winter months instead of planting spinach twice to allow crop rotation.

Table 6: Expected gross margin and profit with recommended plan production in a greenhouse area of 100 m^2 Table

(in BAM)

Description	Greenhouse				
	Control		**Heated**		
	Lettuce	Tomato Pink Rock	Lettuce	Tomato Pink Rock	Spinach
REVENUES	**1,348**	**4,330**	**1,304**	**3,850**	**1,418**
COST					
Seedlings	120	225	120	225	5
Fertiliser	25	190	25	190	6
Pesticide	13	26	28	29	0
Mechanisation	20	30	20	30	20
Labour	218	394	174	530	92
Geothermal pump	0	0	426	13	262
Irrigation	20	20	20	20	10
Supporting material	24	60	24	60	12
Other costs	24	32	42	54	26
TOTAL COSTS	**464**	**977**	**880**	**1,152**	**432**
GROSS MARGIN	**884**	**3,353**	**424**	**2,698**	**986**
Total gross margin	**4,237**		**4,108**		
Depreciation	176		1,415		
PROFIT	**4,060**		**2,693**		

The gross margin in the control greenhouse from lettuce cultivation amounted to 884 BAM, while the gross margin from tomato cultivation was 3,353 BAM. The total gross margin for the control greenhouse was 4,237 BAM. After deducting depreciation costs, the total profit for this greenhouse could amount to 4,060 BAM.
The gross margin from lettuce cultivation in the heated greenhouse amounted to 424 BAM, half of the control greenhouse's. This was primarily due to the electricity costs for heating the greenhouse, which amounted to 426 BAM for lettuce alone. Therefore, the electricity consumption and heating of the greenhouse did not improve the economic viability of lettuce cultivation during the winter period. On the contrary, the results were

twice as poor because the yields were similar, and the selling prices were identical for both greenhouses. The gross margin from Tomato cultivation in heated greenhouses amounts to 2,638 BAM per 100 m². This gross margin is also lower than that achieved in the control greenhouse. The tomato yields were lower in the heated greenhouse compared to the control greenhouse, and labor costs were slightly higher than in the control greenhouse. These two factors contributed to the lower gross margin in the heated greenhouse compared to the control one.
Spinach had a gross margin of 986 BAM, making it the crop with the highest gross margin among those cultivated in the winter period. The total gross margin for the heated greenhouse was 4,108 BAM. After deducting depreciation costs, the total profit was 2,693 BAM. This indicates that the profit achieved in the heated greenhouse, even with the use of a geothermal pump, was significantly lower than in the control greenhouse. Considering that the investments for the heated greenhouse were also much more significant and extensive than for the control greenhouse, it can be concluded that the economic viability and profitability, in this case, proved to be better in the control greenhouse than in the heated one.

CONCLUSION

The study demonstrated that geothermal heating in small-scale greenhouses under the continental climate conditions of Bosnia and Herzegovina offers certain agronomic advantages. Still, these benefits do not justify the high investment and depreciation costs during the observed season. Statistically significant temperature differences were observed between the heated and control greenhouses, particularly during winter's morning and evening hours, which has confirmed alternative hypothesis one. However, this temperature advantage did not translate into consistently higher yields, except in the case of onions. These results rejected the hypothesis of increased yields inside the heated greenhouse. Tomato yields were lower in the heated greenhouse due to temperature stress and fruit deformities caused by suboptimal conditions during the flowering phase. The economic analysis revealed that, despite the potential for an additional crop cycle, the overall profit from the heated greenhouse was lower than that of the control, primarily due to significantly higher depreciation and energy costs.
Therefore, the heated greenhouse was not economically viable under the current market and climate conditions. It is recommended that further research be conducted over multiple seasons to obtain more reliable and representative results.
The significant disadvantage in the experiment was the lack of a double foil in the outside walls of the greenhouse. Such a double foil could ensure better greenhouse insulation, a higher internal temperature, and lower electricity consumption. Furthermore, it is recommended that similar experiments be conducted at this location to confirm or challenge the results obtained in this experiment. As the BiH has few climate zones, comparing the results with the same or similar system in the Mediterranean climate type would be interesting.

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EKONOMSKA EFIKASNOST SISTEMA GEOTERMALNOG GRIJANJA U MALIM PLASTENICIMA U BOSNI I HERCEGOVINI

Sažetak

Proizvodnja povrća u plastenicima nudi mnoge prednosti u odnosu na tradicionalne metode uzgoja na otvorenom. Ove prednosti uključuju regulisanje i kontrolu uslova uzgoja, što dovodi do većih prinosa, proizvodnje tokom cijele godine i povećane efikasnosti raspodjele resursa. Mnoge studije pokazuju da sistemi grijanja poboljšavaju proizvodnju u plastenicima, što dovodi do povećanja prinosa i poboljšane ekonomske isplativosti. Međutim, slična istraživanja nedostaju u Bosni i Hercegovini, posebno u regijama s kontinentalnom klimom. Stoga je osnovni cilj ovog istraživanja bio da se ocijeni ekonomska efikasnost proizvodnje povrća (zelene salete, mladog luka, paradajza i špinata) u plasteniku opremljenom dodatnim sistemom grijanja u odnosu na onaj bez takvog sistema. Eksperiment je izveden u dva plastenika površine 100 m^2 po identičnoj tehnologiji uzgoja.Rezultati su pokazali da su tokom zimskog perioda temperaturne razlike između plastenika bile statistički značajno različite; međutim, posmatrani prinosi nisu pokazali statistički značajnu razliku. Ekonomski rezultati su bili suprotni očekivanjima, sa manjim pokrićima u plasteniku sa grijanjem. Glavni razlog su veći troškovi vezani za amortizaciju (1.238 BAM) i potrošnju električne energije geotermalne pumpe (700 BAM). Zaključno, ovo istraživanje je pokazalo da korištenje geotermalne pumpe nije ekonomski opravdano za proizvodnju povrća, te se preporučuje da se razmotri korištenje ove opreme i u drugim uslovima, kao što su veće površine plastenika s poboljšanom izolacijom (dvoslojna plastika). Nadalje, provođenje sličnih eksperimenata na ovoj lokaciji je potrebno nastaviti kako bi se potvrdili ili osporili rezultati dobiveni u ovom eksperimentu.

Ključne riječi: *ekonomska efikasnost, plastenici, Bosna i Hercegovina, geotermalna pumpa, kontinentalna klima*

AGRICULTURAL POLICY OF BOSNIA AND HERZEGOVINA AND THE CHALLENGES OF EU INTEGRATION PROCESSES*

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Original scientific paper

Abstract

Agriculture in Bosnia and Herzegovina faces numerous challenges, including global crises such as the COVID-19 pandemic and the Russia-Ukraine war, regional issues like African swine fever, and increasingly severe impacts of climate change. On the other hand, BiH is clearly committed to EU integration, as reflected in current state and entity-level strategic documents. Moreover, the country's efforts to accelerate the harmonization of its agricultural policy with the EU Common Agricultural Policy (CAP) have been further motivated by the granting of candidate status in 2022 and negotiation status in 2024 for full EU membership. This paper provides an overview of the development of agricultural policy in BiH from 2014 to 2023 at the state and entity levels (including the Brčko District of BiH) to assess the level of harmonization with the EU CAP using the unique APMC methodology for classifying and visualizing agricultural budget transfers. The analysis covers three groups of measures: (1) market measures and direct support to producers, (2) structural measures and rural development measures, and (3) general agricultural measures. Key macroeconomic indicators such as gross value added, employment levels, and trade balance were analyzed to obtain a comprehensive picture of the current state of the agricultural sector and its significance for the economy of BiH. An important contribution of the paper is comparing Bosnia and Herzegovina's agricultural policy with those of the Western Balkan countries. The main conclusion that can be drawn from this paper is that agriculture is an important sector of Bosnia and Herzegovina's economy, with budgetary transfers to this sector showing a growing trend. However, the process of harmonization with the EU acquis is slow and requires greater decisiveness from policymakers, faster adoption of the legislative framework, and appropriate legal solutions, along with the necessary institutional strengthening to address the current challenges of the EU integration process.

Keywords: *agricultural policy, BiH, EU, CAP, integration processes*

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INTRODUCTION

The agricultural sector represents a key segment of the social and economic life of BiH, given that a significant portion of the population still lives in rural areas and is engaged in agriculture. On the other hand, rural communities in BiH continue to face numerous challenges, including slow productivity growth, high unemployment rates, ongoing depopulation, and fragmentation of agricultural land. Small farms with fragmented plots and low levels of technical and technological equipment are primarily involved in subsistence farming, with minimal surpluses for sale. This results in low productivity, low incomes, small quantities, and questionable quality and consistency of market production (FMAWMF, 2022)

As in most Western Balkan countries, the development of the agricultural sector in BiH is insufficient and is a result of weak value chain organization, lack of modernization and application of new technologies, and a non-functional system for knowledge transfer and innovation to support the sector (Erjavec *et al*., 2021; Kotevska & Martinovska Stojcheska, 2015).

Policy-making in the agri-food sector is a complex process due to the various subsectors, objectives, and behavior of numerous stakeholders, highlighting the multifaceted role of agricultural policy in regulating and supporting actors, as well as in meeting the needs of producers and the wider public. Commitment to the EU path entails stronger investment in improvement, competitiveness, and alignment with standards, which requires significant resources and the attention of policymakers (Erjavec *et al*., 2021). Alignment with the EUCAP is a priority for BiH, as the efficiency of the agricultural sector is crucial for achieving economic and social goals while adhering to environmental standards. All of this underscores the need for greater and bolder engagement by decision-makers in the creation and implementation of agricultural policies at all levels of government in BiH.

Agricultural policy in BiH is implemented at the level of three administrative units: the Federation of BiH, the Republika Srpska, and the Brčko District of BiH. At the state level, the policy primarily focuses on regulating the trade of agricultural and food products and coordinating various relevant areas. Although actual policy-making occurs at the entity level, a national strategic framework is equally important, as it supports BiH's process of EU integration and serves as a prerequisite for accessing EU pre-accession funds.

This is particularly significant considering that, by the end of 2022, BiH became an EU candidate country, and at the beginning of 2024, the EU decided to open accession negotiations with BiH. To enter the process effectively, it is necessary to align with the CAP.

The current EU CAP focuses on renewing the objectives of a smart, resilient, and diverse agricultural sector that ensures food security; enhancing environmental protection and addressing climate change while contributing to the achievement of EU environmental and climate goals; and strengthening the socio-economic fabric of rural areas (Recanati *et al*., 2019).

BiH remains in the early stages of alignment with the EU acquis. Although some progress has been made in agricultural policies and food safety, the country faces significant challenges regarding institutional capacities and legislative harmonization (EC Opinion for 2023). The complex political structure and decentralized governance further hinder alignment with EU standards.
To address these challenges, the governments of both BiH entities have responded by increasing overall budgetary support. Additionally, the competent ministries in both entities are actively considering amendments to agricultural and financial incentive laws, as the current regulations hinder alignment with the EU CAP.
Of particular importance is that AKIS (Agricultural Knowledge and Innovation Systems), FADN/FSDN (Farm Accountancy Data Network), the LEADER approach, and Local Action Groups (LAGs) have not yet been incorporated into agricultural laws at either the BiH or entity levels. These reforms are essential for achieving sustainable agricultural development in BiH, encompassing economic, social, and environmental dimensions.

MATERIALS AND METHODS

To determine the current state of the agricultural sector and its importance to Bosnia and Herzegovina's economy, key macroeconomic indicators were analyzed, including gross value added, employment rates, and agriculture's share in the overall state budget. For a more comprehensive assessment, the level of alignment between Bosnia and Herzegovina's agricultural policy and the European Union's CAP was examined using the unique APMC methodology for classifying and visualizing budgetary transfers in agriculture. The categorization of measures within the APMC database is based on detailed data, including transfer amounts, beneficiaries, implementation criteria, specific requirements, and other relevant information (Rednak & Volk, 2010). The analysis covered three groups of measures: (1) Market measures and direct support for producers, (2) Structural measures and rural development measures, and (3) General measures in agriculture. Additionally, to better understand Bosnia and Herzegovina's agricultural policy, a comparative analysis was conducted with the policies of other Western Balkan countries. The analysis period covered ten years, from 2014 to 2023.

RESULTS AND DISCUSSION

In recent years, agriculture in BiH has been marked by global challenges such as the COVID-19 pandemic, the Russia-Ukraine war, African swine fever (2023 in Republika Srpska), and increasingly pronounced climate change. These factors have led to market disruptions and a worsening trade balance for agricultural and food products, which reached €1.8 million in deficit in 2023 (BHAS, 2024).
Bosnia and Herzegovina's economy has experienced continuous growth since the downturn caused by the COVID-19 pandemic, particularly in 2020, when GDP recorded negative values (-3.1%). Recovery followed, with growth of 7.4% in 2021 and 4.1% in

2022 (CBBH, 2024). The effects of the COVID-19 pandemic underscore the need for a new approach to the agricultural sector, which has gained strategic importance not only economically but also in the context of national security (Coluccia *et al*., 2021).
However, the share of agriculture in Bosnia and Herzegovina's GVA (Gross Value Added) has stagnated in recent years, reflecting the growth of other economic sectors that contribute more significantly to the country's overall economy. In 2017, the agriculture sector accounted for 7.1% of total GVA, but by 2023, this share had declined to 5.2% (BHAS, 2024).
The steady economic growth was accompanied by stable inflation, ranging between -1% and 2% until 2020. However, after that, the country experienced the highest recorded increase in consumer prices, reaching 14.1% in 2022 (CBBH, 2023).

Table 1. Selected general and agricultural statistics in Bosnia and Herzegovina, 2014-2023

Indicator	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
GDP, mill.EUR	13,961	14,592	15,287	16,121	17,100	18,012	17,514	19,084	23,266	25,027
Real growth GDP	1.2	4.3	3.2	3.2	3.8	2.9	-3.0	7.4	4.2	2.2
GVA, mill.EUR	11,487	12,028	12,605	13,293	14,593	15,359	14,735	16,056	19,532	21,020
GDP per capita, EUR	3,959	4,148	4,354	4,601	4,891	5,164	5,040	5,527	6,763	7,303
AgGVA, , mill.EUR	832	910	975	939	1,006	1,008	1,066	1,086	1,125	1,087
Employment (total, 000)	812	822	801	816	822	830	1,173	1,151	1,162	1,196
Employment in Ag (000)	139	147	144	154	129	149	107	108	85	91

Source: SWG BA Statistics base; Central Bank of BiH

Employment in BiH has shown consistent growth, reaching 1.2 million in 2023, while the unemployment rate, based on ILO methodology, dropped to 13.2%. In the agricultural sector, however, the number of employed persons fell to 91,000 (7.6% of total employment), a significant decrease compared to 2013 (155,000 or 18.9%). This decline is not due to modernization but rather the result of negative demographic trends, rural depopulation, and reduced interest in agricultural work.
The situation in the sector, particularly in animal production, shows a trend of decreasing livestock numbers across almost all types and categories of animals in recent years. The introduction of a new methodology for collecting data on total agricultural land use in 2022 revealed a significant difference compared to previous years. Specifically, the total utilized agricultural area in BiH amounted to 534.8 thousand hectares, which is three times smaller than the figure from 2021, when, according to official statistics, the area was 1.87 million hectares. In 2022, BiH adopted a new approach and methodology for collecting agricultural statistical data, aligned with EUROSTAT, marking another step toward EU integration. Additionally, some data,

such as those related to fruit production, are now expressed in standard units of measurement, like hectares, instead of the number of trees. This approach makes Bosnia and Herzegovina's agricultural statistics officially comparable to those of EU countries. However, it should be noted that this has prevented the creation of comparable time series that include the period before 2022. The decreasing production of agricultural and food products is also reflected in the fact that BiH remains a net importer of agricultural and food products, with a constant increase in the trade deficit. In 2023, the deficit reached 1.8 billion euros, with import coverage by exports at only 23.7%. This represents a steady decline compared to previous years, when the coverage was higher—34% in 2017 and 28% in 2021. In 2023, the export of agricultural and food products amounted to 561 million euros, which accounted for 6.6% of Bosnia and Herzegovina's total exports. On the other hand, imports were worth 2.37 billion euros, or 16.7% of the total imports (FTC BiH, 2024).

Due to all the aforementioned factors indicating slow growth in the sector, decision-makers are actively considering changes to existing agricultural and financial support laws, as current regulations significantly slow down the process of alignment with the EU CAP. A particular challenge is the fact that AKIS, FADN/FSDN, the LEADER approach, and LAGs have not yet been included in the Agricultural Law at the level of BiH or its entities. For this reason, through strategic documents, efforts are being made to adopt new guidelines from relevant EU regulations, which entails introducing new measures. Some of these measures include support for local action groups and the LEADER approach, establishing AKIS, enacting new agricultural laws, and creating financial incentives for agriculture. To address these challenges, the governments of both entities in BIH have also responded by increasing overall budgetary support, particularly in 2023.

The following section of the paper presents an analysis of budgetary allocations for the agriculture and rural development sector at the level of administrative units, as well as a comparison of the achieved level of alignment with the CAP with other countries in the Western Balkans.

The total budgetary support in 2023 reached 220.7 million euros, which is 64.4% higher than the previous year (134.3 million euros) and an increase of 154.8% compared to 2019 (86.6 million euros). The main reasons for this increase lie in the significant growth of entity budgets, driven by inflation and higher VAT revenues, as well as the government’s decision to allocate substantial funds to agriculture and rural development. This recognizes the sector as strategically important for strengthening food self-sufficiency, retaining rural populations, and incentivizing interest in agricultural production. An additional reason for the increase in budget transfers is the need to reduce the gap in subsidies compared to neighboring countries and the EU. In addition to the increase, the structure of the total budget for subsidies has also shifted towards sector development, with a notable increase in funding for rural development measures. However, market price policy measures and direct payments to producers still dominate the overall budget transfers, accounting for 64.5% (142.4 million euros). The second level, which includes structural measures and rural development measures, amounts to 66.1 million euros, or 29.9%. General agricultural measures remain the least represented, with only 5.5% (12.2 million euros) of the total agricultural budget.

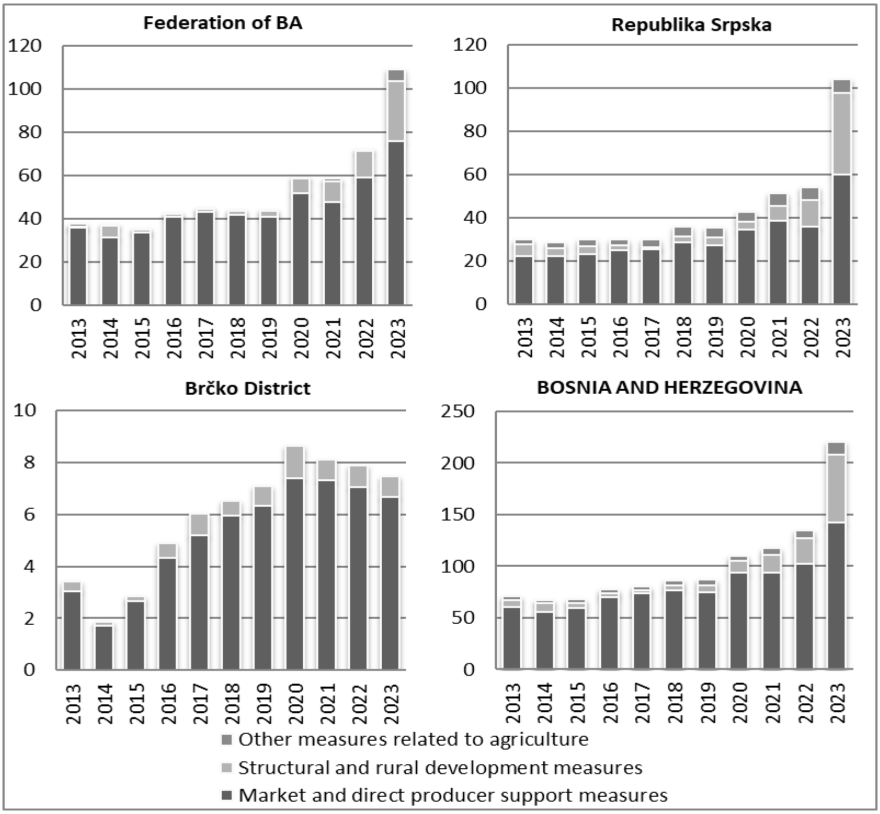


Figure 1: Breakdown of budgetary expenditure for agri-food sector and rural areas, 2013-2023, Bosnia and Herzegovina, in million EUR
Source: BA APMC database

Observed by entities, in 2023, the Federation of BiH recorded a significant increase in its agricultural budget, reaching 108.9 million euros. Total expenditures were 51.1% higher thanin 2022 and 147.9% higher than in 2019, the pre-pandemic year. This significant increase is a response to the challenges posed by the pandemic, as well as the growth of entity revenues and VAT. In the 2023 budget, the largest share (69.6%) is allocated to the first level measures, which include market and direct support to producers, amounting to a total of 75.8 million euros. Structural measures and rural development account for 27.6 million euros, or 25.4% of the budget. General agricultural measures remain neglected, with a modest share of 5.4% of the total agricultural budget of the Federation of BiH.

In the Republika Srpska, the amount of funds allocated for agricultural support in 2023 is 104.3 million euros, which is nearly twice the amount allocated during the pandemic and three times more than in 2019. The structure of budget allocations by pillars of agricultural policy is somewhat more favorable compared to the Federation of BiH. Market and direct support measures for producers account for 57.4%, or 59.9 million euros, while 37.6 million euros is allocated for structural measures and rural development, representing more than a third (36.1%) of the total budget. General agricultural measures are the least represented, with 6.8 million euros, making up 6.5% of the total agricultural budget. In the Brčko District of BiH, the agricultural budget has remained stable over the past three years, ranging between 8 and 8.6 million euros, with 90% of the funds allocated to direct payments.

To gain a better understanding of Bosnia and Herzegovina's agricultural policy, a comparative analysis was conducted with the policies of the Western Balkan countries over a period of ten years, from 2014 to 2023. The analysis primarily focused on the total budget for agriculture and rural development, followed by the share of total budget transfers in GDP, as well as the allocation of budget transfers per hectare and per capita.

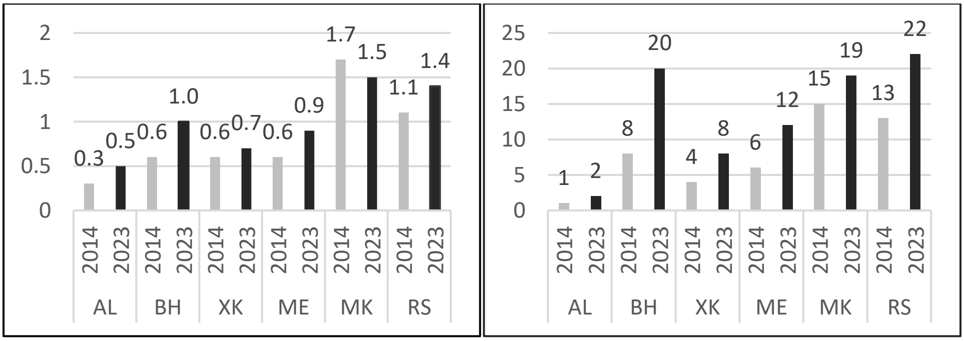
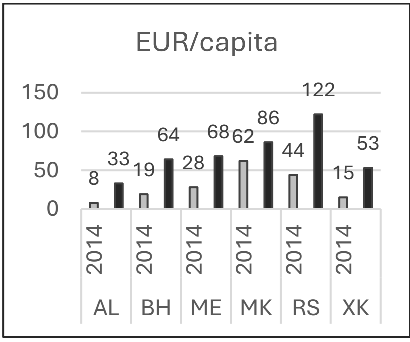


Figure 2. Total budgetary transfers in total GVA (left) and in AgGVA % (right)
Source: European integration of Western Balkan agriculture: state of the art and challenges draft

The proportion of budgetary support provided by WBCs relative to total GVA and agricultural GVA (AgGVA) shows notable variations across countries/territories and over time (2014–2023). In Albania, transfers increased from 0.3% to 0.5% of total GVA and from 1% to 2% of AgGVA (Figure 2). BiH recorded a significant rise, with transfers growing from 0.6% to 1.0% of GVA and from 8% to 20% of AgGVA. Kosovo observed moderate growth, with transfers reaching 0.7% of GVA and 8% of AgGVA. Montenegro saw an increase in agricultural transfers, growing from 6% to 12% of AgGVA. North Macedonia experienced a slight decline in total transfers but showed an increase in agricultural support, rising to 19% of AgGVA. Serbia exhibited the most substantial growth, with total transfers reaching 1.4% of GVA and agricultural transfers soaring to 22% of AgGVA.



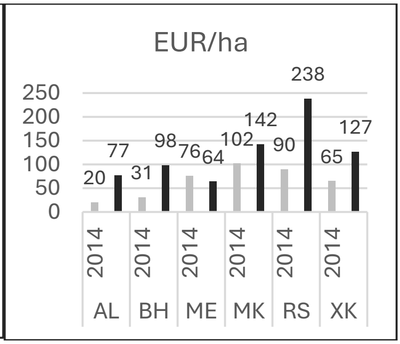


Figure 3. Total budgetary support to agriculture and rural development EUR /ha and EUR per capita
Source: SWG WBC APMC 2024 and WB StatDatabases 2024

There is a clear trend of increasing financial support for agriculture both in BiH and in other Western Balkan countries, highlighting its growing importance in national budgets. Budgetary support per hectare of agricultural land and per capita varies significantly among countries from 2014 to 2023. Albania's transfers rose from EUR 20 to 77 per hectare and from EUR 8 to 33 per capita. BiH increased to EUR 98 per hectare and EUR 64 per capita by 2023. Montenegro saw significant growth, reaching EUR 164 per hectare and EUR 68 per capita. North Macedonia increased to EUR 142 per hectare and EUR 86 per capita, while Serbia recorded the highest growth, reaching EUR 238 per hectare and EUR 122 per capita in 2023. Some WBCs, such as North Macedonia, Montenegro, Kosovo, and Serbia, are nearing EU support levels (EU average: EUR 238 per hectare from direct payments, EUR 340 per hectare from total CAP expenditure). Decision-makers should plan for future CAP developments and adjust national support gradually to align with anticipated EU levels, minimizing potential negative impacts upon joining the Union.

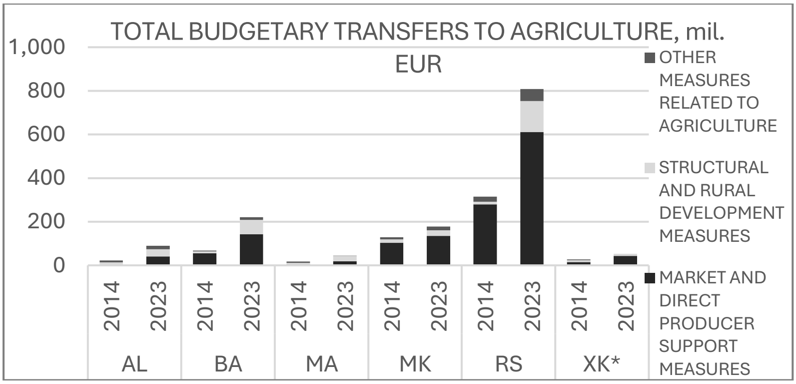


Figure 4. Total budgetary support to agriculture and rural development (million EUR, 2014-2023, nominal terms)

The policy of increasing budgetary allocations for agriculture and rural development is evident across all the observed countries in the Western Balkans. In some countries, these amounts have more than doubled by 2023, particularly in Albania, BiH, and Serbia. However, the majority of the funds are still directed towards market price policy measures (with the exception of North Macedonia). Market support measures have been negligible and only emerged in 2020, exclusively in Montenegro, while in the EU, they make up an average of 5%. Direct support to producers remains a key component of agricultural and rural policy in the Western Balkan countries, particularly in BiH, North Macedonia, and Serbia, which is comparable to the EU-27 average and some neighboring countries such as Bulgaria and Greece. On the other hand, structural and rural development measures have the largest share in Albania, followed by Montenegro and Kosovo. This structure is closer to the model of Adriatic EU countries such as Slovenia and Croatia.

CONCLUSIONS

The legislation in BiH is outdated and currently poses an obstacle to the development of the sector. Therefore, it is essential to update it as soon as possible and align it with EU legislation.

Budgetary transfers for the agricultural sector in both entities of BiH saw an increase during the pandemic period of 2021–2022, driven by the need for additional interventions to support economic recovery. However, the significant rise in allocations in 2023 highlights the recognition of agriculture not only as a vital component of the economy but, above all, as a cornerstone of food security. In 2023, a process of intensified investments in structural measures and rural development initiatives began. This trend should continue, with a gradual increase in funding for these measures by 2027, to establish a balanced allocation between direct payments and rural development.

Structural measures and rural development initiatives are essential instruments for the modernization and long-term development of the agricultural sector.

According to the latest reports from the European Commission on the progress of EU accession countries (EC 2022a-f; EC 2023c-h), the status of chapters remains unchanged compared to previous assessments. North Macedonia, Montenegro, and Serbia have demonstrated moderate preparedness in most chapters, with the only "good preparation" rating still attributed to North Macedonia for Chapter 12. On the other hand, Serbia shows a lower level of preparedness in Chapter 11, while Montenegro lags in Chapter 13. BiH and Kosovo remain at the early stage of preparations for Chapter 13, with BiH also assessed at an early stage of preparedness for Chapter 11. Comparing all countries, it can be concluded that BiH has the lowest level of readiness, highlighting continuous challenges and underscoring the need to accelerate progress in these key segments of the EU accession process.

Continuous investments, strategic allocation of funds, and reforms aimed at modernizing and ensuring the sustainability of the agricultural sector are crucial for improving the socio-economic conditions in the country and aligning with European standards.

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POLJOPRIVREDNA POLITIKA BOSNE I HERCEGOVINE I IZAZOVI EU INTEGRACIONIH PROCESA

Sažetak

Poljoprivreda u Bosni i Hercegovini suočava se s brojnim izazovima, uključujući globalne krize poput pandemije Covid-19 i rusko-ukrajinskog rata, regionalne probleme poput afričke svinjske kuge i sve izraženije negativne posljedice klimatskih promjena. Sa druge strane, BiH je jasnih opredjeljenja za EU integracije što se može vidjeti u aktuelnim državnim i entitetskim strateškim dokumentima, a dodatni razlog bržoj harmonizaciji poljoprivredne politike BiH sa EU Zajedničkom poljoprivrednom politikom (CAP) je dobivanje kandidatskog (2022) i pregovaračkog statusa (2024) BiH za punopravno članstvo u EU.

U ovome radu daje se pregled razvoja poljoprivredne politike u BiH za period 2014.-2023. na državnom i entitetskom (Brčko distrikt BiH) nivou kako bi se utvrdio dostignuti stepen harmonizacije sa EU CAP koristeći se jedinstvenom APMC metodologijom klasifikacje i vizualizacije budžetskih transfera u poljoprivredi. Analiza je obuhvatila tri grupe mjera: (1) tržišne mjere i mjere direktne podrške proizvođačima, (2) strukturne mjere i mjere ruralnog razvoja i (3) opšte mjere u poljoprivredi. Za dobivanje potpune slike aktuelnog stanja poljoprivrednog sektora i njene važnosti za BiH ekonomiju analizirani su ključni makroekonomski pokazatelji, poput bruto dodane vrijednosti, stepena zaposlenosti i vanjsko-trgovinskog bilansa. Važan doprinos rada odnosi se na usporedbu poljoprivredne politike BiH sa zemljama Zapadnog Balkana. Osnovni zaključak koji se može izvesti iz ovog rada je da je poljoprivreda važan sektor

BiH ekonomije, budžetski transferi za ovaj sektor pokazuju trend rasta, ali je proces harmonizacije s pravnom stečevinom EU spor i traži više hrabrosti donosioca odluka i brže donošenje zakonodavnog okvira i adekvatnih zakonskih rješenja uz neophodno institucionalno jačanje koje će biti u kontekstu aktuelnih izazova EU integracionih procesa.

Ključne riječi: *poljoprivredna politika, BiH, EU, CAP, integracioni procesi*

LEGAL ASPECTS OF PROTECTING THE NAMES OF TRADITIONAL FOOD PRODUCTS IN THE EU*

Harun Lozo*[1]

Review article

Abstract

In recent decades, consumers in the European Union have increasingly demanded high-quality food products. Over time, consumers have also shown growing interest in health aspects, as well as the environmental impact of production and animal welfare. For producers, labeling products is the only way to convey information about their characteristics in order to attract consumer attention. To effectively communicate such information, especially in the case of traditional products that meet consumer demands, the legal framework of the European Union provides two labeling instruments: trademark registration and the registration of a geographical indication. A large number of studies have focused on consumer preferences and consumption habits. This paper is motivated by the position of producers who need guidance on product labeling to achieve better market position. The paper focuses on the legal context through an analysis of the requirements for trademark and geographical indication registration, as well as an examination of the legal position of producers after registration. The goal is to determine, through a comparison of the two options, which one offers producers a better legal position, as a broader scope of exclusive rights ensures a better economic position as well. The paper uses legal, analytical, and comparative methods to compare the legal instruments, and it also examines producer preferences through official data on the number of trademark and geographical indication registrations. The results show that the conditions for trademark registration are more liberal, which explains the higher number of trademark registrations. On the other hand, the conditions for registering a geographical indication are stricter, but they provide producers with a broader scope of guaranteed rights compared to a trademark, as well as better implementation of those rights in practice.

Keywords: *traditional food products, legal protection, trademarks, geographical indications*

INTRODUCTION

It is a well-known fact that the choice of food affects physical health. However, food choices are also directly related to mental health, as some studies show that consuming fast food is associated with increased risk of depression in a way that higher

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consumption of fast food may contribute to the development and exacerbation of depression (Rahman *et al.*, 2024). Therefore, consumer demands are increasingly moving in the direction of choosing food products that do not cause harm to the environment, health of people, animals, plants and their welfare (European Union, 2018). Labelling of food products plays a key role in this regard, since labelling is the only way for consumers to verify, during the purchase process or after the consumption, whether a product has been produced according to some specific requirements. (Janssen and Hamm, 2012). Traditional food products often resemble old, well-established production methods in which ingredients and specific *know-how* of producing and processing of final products satisfy such consumer demands. This puts manufacturers in front of a challenge: how to achieve adequate communication with customers and show them that the product contains those elements so that customers are deprived of any worry or discomfort. Also, success in sales results will directly depend on that. The first question to be considered is the definition of tradition and traditional food product. In the Regulation (EC) No 178/2002, as a part of the general legal framework of the European Union regarding food law and procedures in matters of food safety, term "traditional product" is mentioned only as one of the subject matters of its provisions, without specifying broad definition (European Union, 2002). While traditional product is not defined, Article 2. defines "food" as any substance or product, whether processed or not, intendend to be ingested by humans (European Union, 2002). At the same time, another EU regulation, namely Regulation No 1169/2011, establishes the assurance of a high level of consumer protection in relation to food information, taking into account the differences in the perception of consumers and their information needs (European Union, 2011). Although general rules in this regard were given, the notion of tradition and traditional food product was still not defined. This finally happened with the adoption of the Regulation No 2024/1143 which refers to the protection of geographical indications for wine, spirit drinks and agricultural products and where tradition is in the Article 2 defined as proven historical usage of the name of product by producer for a period that allows transmission between generations. This period is to be at least 30 years (European Union, 2024). It follows that, from a legal point of view, labels of food products should contain accurate and objective descriptions whilst only a product whose production has been passed down from generation to generation can be considered traditional. Outside the sphere of legislative definitions, in some studies the definition of tradition and traditional food product was examined from the consumer's point of view, so while the key role in the legal definition is conferred to the time factor and transmission from generation to generation, it was concluded that consumers perceive traditional product from four aspects, as (1) products that are eaten frequently or every day, and are related to health, nature, made on farms without additives, (2) products that have origin from certain locality as tradition can not be exported, (3) products that are part of long-standing cuisines, and (4) products that have distinct taste because "tradition is tasteful" (Guerrero *et al.*, 2009). It is evident that consumers perceive tradition somewhat broader than what is legally defined. Although globalization implies trade without borders, consumers have been paying great attention to the origin of food products for the past several decades. For example, in Switzerland it was determined that even for 82% of consumers the question of product origin is one of the key elements while making a purchase decision (Luykx and Van Ruth, 2008). In such a situation, a large number of studies have been conducted with the aim of determining the preferences and behaviour of consumers. More precisely, there are about 800 academic publications related to the issue of branding and the place of origin of products, the vast majority of which deal with examining consumer behavior and their willingness to pay more for products with proven origin (Florek and Gazda, 2021). This kind of data can make producers anxious as they may find themselves unfamiliar with the paths they need to take in order to properly protect the names of their products and achieve full communication with consumers by conveying the right information about the origin, quality, characteristics or reputation of the products. The aim of this paper is to determine the most effective and optimal modalities of products name protection through the analysis of the applicable legislative in the European Union, which the countries of the Balkan region are trying to harmonize their national legislations for.

MATERIALS AND METHODS

For the purposes of this research, the following legal regulations and databses were used:

- Regulation (EU) 2017/1001 of the European Parliament and of the Council of 14 June 2017 on the European Union trade mark,
- Regulation (EU) 2024/1143 of the European Parliament and of the council of 11 April 2024 on geographical indications for wine, spirit drinks and agricultural products, as well as traditional specialities guaranteed and optional quality terms for agricultural products,
- eSearch plus – EUIPO’s trade mark database,
- eAmbrosia – European Union geographical indications register.

Through the aforementioned materials, an analysis of legal texts on the modalities of protection of product names was carried out, highlighting the advantages and disadvantages for producers of each of them, and special emphasis on the legal consequences and effects of registration. In addition, through the analysis of the number of registrations in databases, general preferences of producers’ protection modality choice were determined. An analytical as well as comparative methods were being used since each modality of protection was analyzed from a legal point of view and different modalities were compared to each other so as to be able to find the best recommendation for producers.

RESULTS AND DISCUSSION

The producer always has a simple option at disposal: not to protect the name of product and leave it to consumers to recognize its qualities or characteristics themselves. But, that would be rather bad choice when the product has some pecularities and consumers are not aware of them. In the literature this is referred as “asymmetric information”, i.e. while producers know whether they have used appropriate ingredients or production methods to achieve the desired level of quality, consumers are deprived of such knowledge because they cannot make any conclusion based on the mere naming of product, or labelling on its packages (McCluskey, 2000). In order to overcome such a gap, in the case of production of a traditional, quality product, it is necessary to enrich its packaging with information and content on the basis of which the average consumer will decide to purchase that product. Each producer is faced mainly with two options: register a trademark or geographical indication. So, what does these options mean, and how do they work in practice?

1. Names protected as trade marks

Most commonly used option is trademark registration. According to the Regulation No. 2017/1001, trade mark is a registered sign that may consist of words, names, designs, letters, numerals, colors, the shape of goods or of the packaging or sounds, if these signs are capable of distinguishing the goods or services of one undertaking from those of other undertakings. (European Union, 2017) Generally speaking, trade marks can be classified into three groups: individual, collective and certification trade marks. (Kur, Dreier, Luginbuehl, 2019)

1.1. Individual trade marks

The first option, i.e. individual trade marks are signs of distinguishing the products or services of one, individual entity or producer. The problem with this option is that, in order to be recognized amongst consumers, producer should gain a huge reputation as being well known for producing high quality food products. This means that there should be a brand established behind the product, for which there are different definitions, but most appropriate one is that a brand represents the totality of the value of a product that includes its name, logo, design, packaging, advertising activities, image or name recognition (Mindrut *et al.,* 2015). In order to achieve that, producer would have to put in a lot of effort, investment, time and consistent practice. Actually, protecting the products name as an individual trade mark in this case seems to be only final step on the road to success. Another shortcoming of this route is the fact that producers would have to be very creative in forming protected signs. In order to convey information to consumers about the product, protected sign should include a description of the product itself, its qualities, origin and the like. According to the Article 7.1. of the Regulation No. 2017/1001, one of the absolute grounds for refusal of trade mark registration is trade mark which consists exclusively of signs or indications which serve to designate the kind, quality, quantity, intended purpose, value, geographical origin or the time of production, or other characteristics of products (European Union, 2017). Rationale of this exception is prohibition of registering of signs, symbols or words that serve to everyone in trade for description of products, as these cannot be monopolized, or reserved for only one producer which would impair or totally disable fair competition on market. Together with other two absolute grounds for refusal of trade mark registration, this means that one of the basic requirements is that the sign should be distinctive, that is, it should not consist of elements that have become customary in the current language or trade (Kur *et al*., 2019). Conveying a message to consumers about the qualities of a traditional food product would require the use of signs that describe the product's characteristics, so these absolute grounds for refusal are the reason why producers have to look for another solution, which is also justified by another reason; according to many surveys, consumers are willing to pay a higher price for traditional and regional products since they associate them with better quality (Angowski *et al*., 2020). In fact, there is a direct link between consumer perception and geographical origin of food product, as consumers evaluate the quality of a product based on its place of origin, transferring opinions about the place onto specific goods that originate therein (Florek and Gazda, 2021). This is the reason why producers need to find solution to register the name of a traditional food product in a way to convey to consumers a message about the qualities, characteristics or geographical origin of the product, and this can definitely not be successfully achieved through individual trademarks. A potential solution is in certification marks.

1.2. Certification trade marks

In contrast to the individual trade mark, in the case of certification mark, according to the Article 83 of Regulation No 2017/1001, a sign can be used to distinguish products which are certified by the proprietor of the mark regarding some pecularities of product which are certified by the proprietor of the mark in respect of material, mode of manufacture of product, quality, accuracy or other characteristics, with the exception of geographical origin, from products which are not so certified (European Union, 2017). This implies the existence of a separate entity as the trade mark owner that verifies that certain product is made of precisely defined ingredients or that product is produced according to precisely prescribed criteria. One example of this type of trademark is "Halal" certificate owned by the Agency for Halal Quality Certification of Bosnia and Herzegovina.[1] This body, as a non-governmental organization, defines the criteria and rules of production that every producer should meet in order to be able to use the protected trade mark. The main specificity of the certification trade mark registration system is that this type of bodies, i.e. owners of the trade mark, do not carry on a business involving the supply of products of the kind certified (European Union, 2017). Simplified, the task of the owner of a trade mark in this case is to define the criteria that the product should meet and to control those properties without being involved in the process of its production or putting it on the market. As for the required criteria of a product, the rules that define them should be submitted to the authority responsible for registration. It can be said that applicants in the process of registration are mostly free to define the criteria of respective products because after the submission of those rules, responsible authorities check only two conditions of general nature, (1) whether rules are contrary to public policy or accepted principles of morality, and (2) whether the

[1] https://halal.ba/znak-halal-kvalitete/ (8.10.2024.)

public is liable to be misled as regards the character or the significance of the mark in a way that it is likely to be taken to be something other than a certification mark (European Union, 2017). However, it is important to emphasize that involvement of public authorities is limited in general because, in addition to the lack of checking the authenticity of the criteria of product, the implementation of prescribed criteria is also not a subject of controls of public authorities. This area of responsibility is again on the side of the owner of the certification trade mark, that is the only one empowered to bring an action for infringement, as any other person can do it only with specific authorization from the owner (European Union, 2017). Not only the owner of the certification trade mark, but also all its potential and actual users, this, similar to the case of an individual trade mark, puts in the position that, through their private initiative, long-term and consistent work and investing a lot of efforts and costs, they have to build recognition amongst consumers. Also, according to surveys, there is clear tendency that consumers show more trust for food information when such information are provided by public authorities or entities that are closer to the public sector (Nocella *et al.,* 2014). Similarly, consumers also trust labels on food products whose authenticity is guaranteed by scientific experts more than labels backed by producers themselves (Wu *et al*., 2021). There are other criteria on the basis of which consumers show trust in labels, e.g. the large number of different labels or quality schemes on food products on the market leads to consumers simply having less trust (Sayogo *et al*., 2016).

For producers, understanding which type of labels consumers trust most is crucial. Key insights on this matter are highlighted in the consumer awareness survey conducted by the European Union/Eurobarometer in 2022, which included respondents from all member states totaling 26.509 participants (European Food Safety Authority, 2022). The highest level of trust, at 89%, is placed in general practitioners and specialist doctors. As for the relationship of trust towards public authorities and the food industry, European Union institutions and national authorities are trusted by 66% of respondents, while the food industry receives the trust of only 45% of consumers. This clearly shows that in order to convey a message about the values and qualities of food products, producers should focus on providing information through channels associated with public authorities.

However, there is no prohibition for the certification mark to be registered by a public body, but in practice, according to the data from the Registry of the European Union Intellectual Property Office (hereinafter: EUIPO), this is extremely rare.[2] Similar to the case of individual trade mark, element that is unfavorable for producers is prohibition on registering the names of the certain geographical area as certification trade mark which prevents consumers from creating an image about the total quality and uniqueness of a certain geographical area and the product that originates therein. Another unfavorable element for producers is still applicable prohibition of registering descriptive signs that describe the product and its characteristics, so the certification

[2] On October 8, 2024, 654 certification marks were registered in the EUIPO database https://euipo.europa.eu/eSearch/#advanced/trademarks/1/100/n1=KindMark&v1=%22EU%20Certificate%22&o1=AND&sf=ApplicationNumber&so=asc

trade mark should include a sign that is distinctive and unique. Taking into account all the results of the analysis of legislative rules, the certification mark is suitable for registration when there is already an established quality standard from the third party, so producers decide to join that quality scheme and thus convey to consumers the message that their traditional product is enriched with what the certification mark criteria require. Registering a new certification mark containing a unique sign would require a long time for convincing consumers of the quality of their product, because such a sign would be unknown to consumers until then. Shortcomings of the certification mark system, which do not allow the registration of names of geographical locations, have been corrected to some extent by another specific type of mark, which is a collective trade mark.

1.3. Collective trade marks

As another specific type of trademark, collective trade mark shares more similarities with the individual than with the certification trade mark. According to the legal definition in the Article 74.1. Regulation No. 2017/1001, collective trade mark consists of a sign that is capable of distinguishing the goods or services of the members of the association which is the proprietor of the mark from those of other undertakings (European Union, 2017). In this case there is an association as a legal form consisting of several individual entities or producers of traditional food products. Association itself should be organized in such a way that is capable to have rights and obligations of all kinds, e.g. to make contracts, accomplish other legal acts, to sue and be sued (European Union, 2017). Therefore, by registering a collective trade mark, several producers of traditional food products join forces to convey the message that it is a product of their group, with some characteristics or qualities that are specific only to that group of producers. The similarity of this type of mark with the certification mark is that the group of producers defines rules by which it determines the conditions of who can be their member. As a part of these rules, group of producers can also prescribe criteria that the product has to meet in order for the producer to become a member of the group and use the collective trade mark. But, in contrast to the certification mark where the holder of the mark may not engage in the activity to which the mark refers, here the members of the association, that is the holder of the trade mark, are also producers of products to which the mark refers. If we take into account the lack of trust of consumers towards information on labels provided by private entities compared to public bodies as previously pointed out in the paper, this can be problematic for conveying the message to consumers about the characteristics of a traditional product. With the certification trade mark, there is an independent certification body whose only task is to check whether the product complies with the given criteria. With a collective trade mark, there is no such intermediary. For this reason, the risk of lack of consumer confidence with collective marks is greater than with certification marks, because producers themselves define the criteria and check their compliance. Unlike the certification trade mark, with the collective trade mark a sign that indicates the geographical origin of a product can be registered, which is one of the most significant feature of this type of trade mark

(European Union, 2017). However, this possibility is not without limitations as the registration of a geographical name cannot prohibit third party from using in the course of trade such names or indications if the usage is in accordance with honest practices (European Union, 2017), This can in a certain way overcome the potential risk of lack of consumer confidence in conveying a message about a traditional product through a collective trade mark, as it gives consumers the opportunity to link the perception of a certain geographical location to the qualities or characteristics of a traditional food product that originate from there. Even in this case, producers will define criteria, i.e. ingredients and production methods, so consumers can rely on the quality and special characteristics by trusting producers, who, if they do not act according to honest practices, can abuse their position. In addition, the literature highlights the viewpoints that are supported by the explanations of the judgments of the Court of Justice of the European Union that the main purpose of using geographical indications in the collective trade mark is to mark the commercial and not the actual, geographical origin of the product (Pila, 2022). In relation to general rules above mentioned according to which producers determine *code of practice*, collective trade mark system has rather limited range in authentically conveying information to consumers about traditional product and its pecularities which could lead them further to make better purchase decisions.

Considering these two modalities, a need for objective, impartial and authentic way of conveying information about products clearly emerges. This channel of communication should be realized through the structure of public bodies or entities whom consumers turst the most. Environment of liberal economy may not be the best place to expect private initiatives to be fully driven by public policy. But there is one exception that can be said to be a part of the identity that the European Union jealously guards, and that is geographical indications.

2. *Names protected as geographical indications*

At the level of the European Union, legal framework for the protection of geographical indications for foodstuff and agricultural products was established for the first time in 1992 adopting Regulation No 2081/92. In the preamble of this regulation it is clearly stated that even at that time consumers were paying more attention to the quality of food products rather than quantity, which is why the demand for products with proven geographical origin was growing. For that reason, one of the goals of policy was to give consumers clear and succint information regarding the origin of the product (European Economic Community, 1992). It is clear that conveying information about the qualities of the product is closely related to the geographical location, so instead of presenting a lot of confusing information, it is enough to indicate that the product originates from a certain place, which will be enough information for consumers. For example, it is enough to say “Parmigiano Reggiano” and everything is clear even for less informed consumers. In addition to conveying information to consumers about product qualities, this legal framework also had the task of promoting agricultural production and rural develompent (European Economic Community, 1992). In the recently adopted

Regulation No 2024/1143, these goals remained almost the same. As emphasized in paragraph 7 of the Preamble, consumers still demand quality products with specific characteristics which are attributable to their origin (European Union, 2024). Besides the aim of supporting policies of the Common Agricultural Policy, there are also goals related to sustainability, European Green Deal and circular economy, but elaborating these issues would go beyond the scope of this paper. It is important to keep in mind that conveying information to consumers about product qualities is one of the main objectives of the legal framework for the protection of geographical indications, which is actually a question which an answer is sought for in the context of producer and their position. As it is not possible to use any descriptive terms while registering trade mark, and with the registration of the name of geographical location, with which consumers associate quality and characteristics of a product, this limitation is not present, it is necessary to carry out an analysis of the definition and concepts of gegoraphical indications. In the legal framework of the European Union, legal protection is ensured through two modalities: protected designation of origin (hereinafter: PDO) and protected geographical indication (hereinafter: PGI).

2.1. PDO vs. PGI – is there any difference at all?

PDO is a name of an agricultural product which identifies a product originating in the specific place, whose quality or characteristics are essentailly or exclusively due to a particular geographical environment with its inherent natural and human factors, and the production steps of which all take place in the defined geographical area (European Union, 2024). Precondition that a product derives its quality or characteristics *essentially or exclusively* from the geographical environment is based on the French concept of *terroir* (Kur *et al*., 2019), which refers to interactions of a certain geographical area and its natural and biological factors such as climate, soil, endemic plant varieties or animal species together with production practices, which ultimately lead to an influence on the qualities or characteristics of the product (Ballantyne *et al*., 2019; Clark and Kerr, 2017). On the other hand, the PGI contains somewhat looser requirements for registration as PGI is a name which identifies a product originating in a specific place, whose given quality, reputation or other characteristic is essentially attributable to its geographical origin and at least one of the production steps of which takes place in the defined geographical area (European Union, 2024). There are three main differences between PGI and PDO, (1) with PGI, the qualities and characteristics of the product can essentially be attributed to the geographical location, while with PDO this condition is stricter, as they should be a consequence of essential or exclusive impact, (2) besides quality and characteristic, reputation is standalone ground for registration, and regardless of whether it is about some qualities or characteristics, registration will be possible if the product has gained a reputation among consumers, (3) with PGI, it is sufficent that only one production step takes place in a certain geographical area, while with PDO, all production phases should take place in defined area. The right of geographical indication is specific in its nature, it is a collective right as referred in literature, because the request for registration of the name can only be submitted, incuding some exceptions, by a group of producers, who defined the product specification, so all producers who respect the prescribed criteria are allowed to use the protected name, regardless of the number of producers. This is a bit reminiscent of the system that applies to the collective trade mark, because even in this case, producers are the ones who define the product criteria. But, as pointed out with the collective mark, the problem that exists is the lack of consumer trust, because consumers are less likely to trust information provided by private entities, they would rather be informed by information provided or verified by public authorities. And this is exactly where geographical indications come into play and satisfy both producers and consumers. The procedure for registration both PDO and PGI is conducted in two stages, first at the national level of the member state of the European Union or third country, and then at the level of the European Union. In the national phase of the procedure, according to Article 10.3. of Regulation No 2024/1143, it is examined by the competent public authorities whether the application for registration meets the prescribed conditions for registration (European Union, 2024). Also, the check by a public body, that is, by the European Commission, is also carried out in the phase of the procedure at the Union level, so the registration is to be checked and verified twice by public bodies, one of them being the European Commission itself. It finally may solve all consumer concerns, and at the same time serve as a very important guide for the producers themselves. As consumer trust is ensured in this way, the next question is how to convey the message to consumers about special qualities, characteristics or reputation of the product. It is necessary that consumers, by looking at the product label, can easily be informed that it is a protected product name whose pecularities have been checked and verified by competent public authorities. In this regard, an effective solution has been reached in the Regulation No 2024/1143, which established special symbols designed to mark and publicize both PDO and PGI (Figure 1), and for the case of agricultural or food products originating in the Union, labelling products with these symbols is mandatory (European Union, 2024).

Figure 1. Symbols of PDO and PGI[1]

Producers from third countries can also take advantage of this benefit because they can, albeit without obligation, label their products with these symbols if the names are

[1] https://agriculture.ec.europa.eu/farming/geographical-indications-and-quality-schemes/geographical-indications-and-quality-schemes-explained_en (10.10.2024.)

registered at the level of the European Union (European Union, 2024). Before the adoption of Regulation No 2024/1143, there was a process of evaluation and impact assessment in which numerous studies were conducted by the European Commission. One of them included the question of recognition of the symbol by consumers and it was determined that only 20% of consumers recognize the PGI symbol while about 14% of them recognize the PDO symbol (European Commisson, 2021). Therefore, the European Commission, recognizing this as a problem, is determined to undertake a series of measures and activities to increase consumer awareness of the meaning of these symbols (European Commission, 2022). This is also one of the objectives of Regulation No 2024/1143, where in paragraph (20) of Preamble it is stated that system of the protection of geographical indications should, in general, significantly contribute to increased awareness, recognition and consumer understanding of the symbols (European Union, 2024). Despite the obvious lack, according to which the actual level of awareness among consumers is relatively low and not satisfactory, this registration option is still tempting for producers. The reason is simple – in the case of trademarks, they would have to make their own efforts so that the trademarks would be recognizable among consumers. However, in the case of geographical indications, the European Union with its administrative apparatus does this for them and will continue to do so in the future, which is expected to be bright because consumer awareness will be much higher in it. Involvement of competent public bodies in the registration process is not the only advantage of the system of protection of geographical indications over trade marks. Such advantages also exist in other segments, such as the scope of legal protection and the enforcement of rights in practice.

2.2. Broad protection and enforcement ex officio

Scope of protection by which producers are protected in case of registration of PDO or PGI is quite broad. For the purposes of this paper, it is sufficient to point out that protection includes the prohibition of any misuse, imitation or evocation even if the true origin is indicated, or if the protected name is translated, transcribed or is accompanied by an expression such as “style”, “type”, “method”, “as produced in”, “imitation”, “flavour”, “like” or similar (European Union, 2024). In fact, protection includes any form of abuse or use that endangers the registered name or damages its reputation or causes any damage to it, so only and exclusively manufacturers who produce in accordance with the product specification can use the protected name. This scope of protection is granted regardless of whether the misuse of the name misleads consumers, which makes this form of protection absolute in nature (Song ans Wang, 2022). On the other hand, the scope of trade mark protection is narrower, so there is a prohibition to use a sign that is identical to registered sign in relation to identical products which the trade mark is registered for. Beyond this scope, i.e. when there is not identical but similar sign and similar products, protection would be available only if there exists a likelihood of confusion on the part of the public (European Union, 2017). This type of protection which is conditional on misleading consumers is referred as relative protection (Hasić and Rački Marinković, 2022). Another and perhaps the most important segment that indicates the greater effectiveness of the system of geographical indications compared to trademarks is the enforcement of rights. According to the provision of the Regulation No 2024/1143, after the name is registered, the competent authorities of the member states are obliged to carry out controls and inspections on the market in order to suppress abuses and to exclude from sale all those products that are illegally labeled with the registered name (European Union, 2024). This method of *ex officio* controls and inspection is actually an additional tool, because right holders in member states can enforce their rights through regular civil proceedings. On the contrary, in order to enforce the right arising from the trademark, it is necessary for the holder of the right to submit an initiative, i.e. to request protection either through the court or any other available legal instrument (European Union, 2017). This further means that they need to actively monitor the market and establish some kind of "surveillance of competitors activities" in order to check if there is any potential violation of their rights, which means an additional burden for them, so they are in a much better position when, instead of a registering trade mark, they register a PDO or PGI. It seems that, from a legal point of view, it becomes quite clear which registration modality would be more effective for producers. However, in order to gain a complete perception, it is necessary to take into account the number of registrations of individual protection modalities in order to establish what the preferences of producers were in the past period.

3. Registration numbers

For the purposes of this paper, an analysis of the contents of the EUIPO database on registered trade marks[1] was performed. The search was carried out according to the Nice Classification[2] of products, with regard to three classes, class number 29 (meat, fish, poultry and game)[3], number 30 (coffee, tea, cocoa and artificial coffee)[4], and number 31 (raw and unprocessed agricultural, aquacultural, horticultural and forestry products)[5], with results shown in the Table 1.[6] Since a product class is chosen for each registration, some applicants choose only one, two, three or more classes. The first three columns represent the number of registrations where only one class was selected, while the fourth column represents the number of registrations where all three classes were selected cumulatively.

Table 1. Number of trademark registrations by product classes

Class 29	*Class 30*	*Class 31*	*Class 29, 30, 31*
15.054	183.092	85.652	24.816

[1] https://euipo.europa.eu/eSearch/
[2] The Nice Classification is an international classification of goods and services applied for the registration of marks. The whole sistem is classified into 34 categories of products, https://www.wipo.int/classifications/nice/en/ (14.10.2024.)
[3] Meat, fish, fruit, vegetable-based food, edible insects, milk beverages, milk substitutes, preserved mushrooms, pulses and nuts or seeds prepared for human consumption.
[4] Beverages with coffee, cocoa, chocolate or tea base, cereals prepared for human consumption, pizza, pies, sandwiches, chocolate-coated nuts, flavourings, other than essential oils.
[5] Unprocessed cereals, fresh fruits and vegetables, plant residue, unprocessed algae, unsawn timber, fertilized eggs for hatching, fresh mushrooms and truffles, litter for animals.
[6] The results contain number of trade marks registered on 14th October 2024.

Class number 30 contains the largest number of registrations, while there are fewest in class 29, which actually contains the most types of products that could correspond to the description of traditional food products. When it comes to classification into three categories: individual, collective and certification trade marks, an analysis was performed for all three classes of products together, and the results are shown in the Table 2.

Table 2. Number of product registrations by classes and categories of trade marks

	Individual TMs	*Certification TMs*	*Collective TMs*
Class 29	149.309	154	570
Class 30	182.584	123	364
Class 31	85.107	140	387
Class 29, 30, 31	24.505	92	203

It is evident that the largest number of trademark registrations related to food products refer to the individual trade marks. The numbers are far lower in the category of certification or collective marks, which have a greater potential to convey a message to consumers about product qualities, characteristics or reputation.
When in comes to geographical indications, the situation is significantly different, so the analysis of the eAmbrosia database[1] of the European Commission determined the number of registrations[2] classified by PDO and PGI, shown in Table 3.

Table 3. Number of registered PDO and PGI in the European Union registry

PDO	PGI	Total
728	990	1.718

These results show that there is a far lower number of registrations of geographical indications compared to individual trade marks. However, another correlation is also interresting, namely between the certification and collective trade marks on the one hand and geographical indications at the other. The total sum of all certification and collective trade marks in all four options (when only class 29, 30 or 31 was selected and when all classes were selected together) is 2.033, while the total number of registered geographical indications is 1.718.
This shows a clear tendency: producers still prefer trade marks over geographical indications which refers mostly on individual trade marks. When it comes to certification or collective trade marks as special modalities of conveying a message to consumers, the figures are close to equal.
After the analysis of the relevant provisions from the legislation of the European Union, and also after the analysis of some data related to the number of registrations, several conclusions and recommendations for future research can be presented

[1] https://ec.europa.eu/agriculture/eambrosia/geographical-indications-register/
[2] The results contain number of GIs registered on 14th October 2024.

CONCLUSIONS

Once a particular food product has been determined to meet the criteria for "traditional product", there are several routes to registering its name and informing consumers that it meets their specific requirements. The first and simplest of them is the registration of an individual trade mark, which is not recommended especially for small producers, since it will be difficult for any message to reach consumers. Also, it is possible to register a certification or collective trade mark, which is associated with certain disadvantages such as a lack of consumer confidence in the authenticity of information conveyed, the need for the producers themselves to take care of the enforcement of the right, and relative protection of the right conferred. Therefore, for any product that meets the criteria to be described as "traditional", and whenever possible, registration of a geographical indication should be taken into consideration. This registration modality not only removes the problem of lack of confidence among consumers, but also enables producers to easily enforce their rights with the assistance of the administrative apparatus, with very broad scope of absolute protection. It seems that such recommendations were not followed by producers in practice. This is confirmed by the large number of individual trade marks in food sector, followed by certification and collective trade marks, and the lowest number in the category of geographical indications. Regardless of the number of registrations, it is evident that the registration of names of traditional food products as geographical indication (PDO or PGI) is the most effective route of protection. The limitation of this research is that due to the scope of the paper, it was not possible to perform an analysis of individual products for which certification or collective trade marks were registered, in order to determine which of them fulfills criteria of a traditional product. It is also a recommendation for future research with aim of proposing to shift a status of registration from trade mark to geographical indication for particular products, which would significantly improve the status of the product itself, as well as the legal and economic position of the producer.

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PRAVNI ASPEKTI ZAŠTITE NAZIVA TRADICIONALNIH PREHRAMBENIH PROIZVODA U EU

Sažetak

Među potrošačima u Evropskoj uniji su posljednjih nekoliko decenija prisutni zahtjevi za kvalitetnim prehrambenim proizvodima. Vremenom, potrošači sve više interesovanja pokazuju i za zdravstvene aspekte, te utjecaj proizvodnje na okoliš i dobrobit životinja. Proizvođačima je označavanje proizvoda jedini način da se prenesu informacije o njihovim karakteristikama, kako bi privukli pažnju potrošača. Da bi se uspješno prenijele takve informacije, pogotovo kada su u pitanju tradicionalni proizvodi koji udovoljavaju zahtjevima potrošača, u pravnom okviru Evropske unije postoje dva pravna instrumenta označavanja i to registracija žiga i registracija oznake geografskog porijekla. Veliki broj provedenih istraživanja se odnosi na preferencije potrošača i potrošačke navike, pa je ovaj rad motivisan položajem proizvođača kojima je neophodno dati smjernice za označavanje proizvoda radi što boljeg tržišnog plasmana. Rad je usmjeren na pravni kontekst kroz analizu uslova za registraciju žiga i oznake geografskog porijekla, te analizu pravnog položaja proizvođača nakon registracije. Cilj je da se komparacijom jedne i druge opcije utvrdi koja od njih proizvođačima garantuje bolji pravni položaj jer je širi obim isključivih prava garant bolje i ekonomske pozicije. U radu se pravnim, analitičkim i komparativnim metodom vrši uporedba pravnih instituta, a uz to se ispituju i preferencije proizvođača u dosadašnjem periodu kroz zvanične podatke o brojevima registracija žiga i oznaka geografskog porijekla. Rezultati pokazuju da su uslovi za registraciju žiga liberalniji, zbog čega je evidentan i veći broj registracija žiga. Sa druge strane, uslovi za registraciju oznake geografskog porijekla su striktniji, ali proizvođačima garantuju veći obim zagarantovanih prava u odnosu na žig, te njihovu bolju provedbu i ostvarivanje u praksi.

Ključne riječi: *tradicionalni prehrambeni proizvodi, pravna zaštita, žigovi, oznake geografskog porijekla*

THE IMPACT OF DIFFERENT DRYING METHODS ON SELECTED QUALITY PARAMETERS OF CELERY ROOT

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Original scientific paper

Abstract

In present study, the impact of different drying methods (convective drying, lyophilization and combined drying) on the color, sensory and antioxidative attributes of celery root, was investigated. Samples of celery root were dried convectively in a dryer at 50°C to a constant mass, in a lyophilizer for 48 hours, and by combined dehydration that included low-energy osmotic pretreatment in sugar beet molasses and shortened consecutive lyophilization for 24 hours. Subsequently, dehydrated samples were powdered and subjected to the color instrumental analysis (L, a, b coordinates), sensory analysis (color intensity, taste and odor) and analysis of total phenols content and antioxidant capacity (determined by ABTS and DPPH methods). The results showed that convectively dried celery root sample was darker than combinedly dried, with a greater share of red and yellow tones. The greatest departure from usual taste and odor (grade 4) was characterized for convectively dried powder (grade 5.5 and 5.9), followed by combinedly dehydrated celery (grade 3.2 and 3.0). Regarding the impact of the drying type on the antioxidant capacity, the combined drying confirmed to be the most efficient, DPPH 163.28 μmol TE/100 g d.m. and ABTS 577.28 μmol TE/100 g d.m., due to the molasses input (an excellent source of antioxidants) in the dehydrated celery root. The powder of celery root previously osmotically dried in molasses had an 8.5% higher content of total phenols in comparison to lyophilized powder, even though lyophilization is one of the most effective methods in preserving phenols.

Keywords: *Combined dehydration, Lyophilization, Osmodehydration, Celery root, Antioxidant capacity*

INTRODUCTION

Celery (*Apium graveolens* L. var. *rapaceum*), an aromatic vegetable, abounds in health-promoting compounds such as dietary fibers, minerals, vitamins and essential oils (Golubkina *et al.*, 2020; Singh *et al.*, 2023). Numerous studies have highlighted

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pronounced antioxidant properties due to the presence of phenols compounds (apigenin, luteolin, quercetin, ferulic acid, kaempferol, p-coumaric acid etc.) in celery root (Beltrán Sanahuja *et al.*, 2021; Pajević *et al.*, 2021). Celery root is used raw or cooked in everyday food, but its applicability in the food processing is limited due to its specific aromatic properties (Szarek *et al.*, 2024).

The high temperatures used in convective drying, a procedure common in the vegetable industry, lead to a reduction in the quality of the dried product, among which the most noticeable are loss of colour, change in texture, impair the flavour and loss of nutrients (Kręcisz *et al.*, 2023). Application of lower temperatures, such as lyophilization, creates a good basis for maintaining the nutritional and sensory dried products' properties, but it requires a lot of energy, and simultaneously the costs of the process and the process' duration are increased (Marić *et al.*, 2020; Ignaczak *et al.*, 2023). Therefore, a good solution in terms of economic and energy savings is energy-undemanding osmotic dehydration as a pre-treatment in combination with shortened lyophilization of high energy consumption proposed in the research of Filipović *et al.* (2022).

The increasing focus on utilizing side products from the food industry, like sugar beet molasses, highlights their potential as sources of natural bioactive compounds. These components might enrich foods, offering both nutritional benefits and ecological advantages (Molina-Cortés *et al.*, 2020; Shafia-Atikah *et al.*, 2020). One practical application of molasses is in osmotic dehydration, a process that is both energy-efficient and simple. Soaking fresh vegetables in concentrated molasses, allows the water to be drawn out of the vegetables while simultaneously allowing beneficial components from the molasses to be absorbed (Cvetković *et al.*, 2019; Filipović *et al.*, 2022).

The main objective of this research was to examine the effect of three different dehydration methods, the usual convective drying and lyophilization and the innovative combined drying procedure of osmodehydration in molasses and successive lyophilization, on color, sensory and antioxidant properties of celery root.

MATERIALS AND METHODS

Material used

Fresh celery root (*Apium graveolens* L. var. *rapaceum*, Alabaster variety) was bought in a grocery store in Novi Sad, Serbia. The celery root contained an average dry matter content of 9.25%, total phenols content was 860.56 mg GAE/100 g d.m. and the determined antioxidant activities were 147.75 μmol TE/100 g d.m. for DDPH method and 576.57 μmol TE/100 g d.m. for ABTS method. The sugar beet molasses, utilized as a solution in the osmotic pre-treatment process, was sourced from a sugar factory (Crvenka, Serbia) and with the total dry matter content of 86.24%. Fresh celery root samples were prepared in the same way for all three dehydration methods: washed under tap water, dried with a towel, peeled and cut with a knife into cubes measuring 1 cm on each side.

Convective drying

Drying of diced celery root samples to constant weight at 50°C was performed using a convective dryer (Instrumentaria, Zagreb, Croatia), after which they were pulverized used a universal laboratory mill (Solem, ZBPP, Bydgszcz, Poland) to create celery powder.

Lyophylization

In this drying procedure, fresh diced celery root samples were frozen at -30°C before being subjected to lyophilization with an apparatus (Christ ALPHA1-2 LDPLUS, Osterode am Harz, Germany). The settings for freeze-drying process included a pressure of 1.6 Pa, a condenser temperature of -57°C, over a processing time of 48 hours. Following this step, the dried samples were ground into a powder using a laboratory mill.

Combined drying

This method was conducted in two phases: osmodehydration in molasses, followed by the lyophilization process. The celery root cubes were immersed in vessels containing molasses (1:5 ratio). Osmotic dehydration occurred for 5 hours at atmospheric pressure and room temperature. After this period, the osmotreated celery root samples were removed from the osmotic solution, rinsed with tap water to eliminate excessive molasses from the surface, and patted with absorbent paper to absorb additional moisture. The osmodehydrated samples were then frozen and stored overnight, before undergoing lyophilization using same device. The lyophilization parameters were the same as in previous procedure, but the drying time was shortened to 24 hours. After this time, dried celery root samples were ground.

Total phenols content and antioxidant capacity determination

To determine the total phenols content and antioxidant capacity of dried celery powder, a mixture of 2.5 mL of ethanol, acetic acid, and water (in a ratio of 50:8:42) was combined with 500 mg of pulverized sample and mixed for 2 minutes on a vortex. The mixture was then centrifuged in duration of 3 minutes at 12,000 rpm. The liquid phase was separated and subsenquently filtered using a 0.45 μm filter.
Total phenols content in these extracts was measured by the Folin-Ciocalteau method, as described in the paper Nićetin *et al*. (2024). The results were calculated using a calibration curve and expressed as milligrams of gallic acid equivalents in 100 grams of dry matter. Antioxidant capacity was assessed using two methods for free radical scavenging: the DPPH assay according to Šaponjac *et al*. (2016) and the ABTS assay according to Aborus *et al*. (2018). The results were calculated by using appropriate calibration curves and expressed as micromole Trolox equivalents per 100 grams of dry matter.

Color determination

The color attributes of the samples were evaluated in six replications, using the device Chroma Meter (CR-400, Konica Minolta, Tokyo, Japan) with a contact surface diameter of 8 mm. Calibration was done in advance, using a white refrence standard. The color analysis' results were displayed in accordance with the CIElab color system, which implies the following coordinate definitions: L denotes brightness with values from 0 (black) to 100 (white), a mesures greenness/redness (-a for green to +a for red), and b blueness/yellowness (-b for blue to +b for yellow) (Šobot *et al.*, 2019).

Sensory evaluation

A panel of ten evaluators was established in accorance with the ISO 6658:2017 standard. Determining descriptors for sensory evaluation of dried celery root was initially conducted by the lead evaluator and later refined by the other panelists. The final list included three descriptors: one for color intensity and two for deviations from the standard taste and odor. A seven-point scale was utilized, with 1 representing the lowest score and 7 the highest for color intensity. The optimal score for taste and odor were established to be 4.

Sensory testing of the samples was conducted in the laboratory of the Institute of Food Technology in Novi Sad, Serbia, following the ISO 8589:2007 standard. Dried samples were offered to the panelists on white plastic trays, each labeled with a randomly assigned three-digit code. The panelists were received water to cleanse their mouths between each tasting.

Statistical analysis

The significance of differences across all quality responses of the three different dried celery root samples was evaluated using analysis of variance (ANOVA). STATISTICA 12.0 software (2013) (StatSoft Europe, Hamburg, Germany) was used for ANOVA analysis.

Principal component analysis (PCA) was applied in order to charaterize and differentiate tested samples and their responses. PCA calculation was performed using Microsoft Excel ver. 2016. (Microsoft Corporation, Redmond, WA, USA), with the XLSTAT Version 2014 5.03 Add-in (Lumivero, Denver, CO, USA).

RESULTS AND DISCUSSION

The obtained results for chemical (total phenols content and antioxidant capacity), physical (color) and sensory changes (color intensity, taste, odor) in celery root depending on the applied drying method, are shown in Table 1. The results indicate that the drying method had a statistically significant effect on all analyzed parameters of dried celery root.

Table 1. The effect of different drying methods on celery root' phenols content, antioxidant capacity, colour and sensory parameters

	Convective drying	**Lyophilization**	**Combined drying**
Total phenols content (mg GAE/100 g d.m.)	246.47±1.81^{a}	820.41±6.78^{b}	890.14±7.63^{c}
Antioxidant capacity by DPPH method (μmol TE/100 g d.m.)	42.37±0.34^{a}	141.21±1.41^{b}	163.26±1.58^{c}
Antioxidant capacity by ABTS method (μmol TE/100 g d.m.)	168.91±0.84^{a}	563.27±2.09^{b}	577.28±6.63^{c}
Brightness (L)	41.69±0.27^{a}	79.57±0.63^{c}	54.10±0.28^{b}
redness (+a)	8.56±0.04^{c}	3.54±0.03^{a}	6.07±0.06^{b}
yellowness (+b)	19.76±0.15^{b}	27.64±0.53^{c}	16.86±0.14^{a}
Color intensity	4.72±0.04^{b}	3.10±0.06^{a}	5.30±0.08^{c}
Taste	5.5±0.05^{c}	4±0.03^{b}	3.20±0.06^{a}
Odor	5.9±0.02^{c}	4±0.07^{b}	3.00±0.02^{a}

*Results are presented as average value ± standard deviation, $^{a–c}$ Different letters in superscript of the same table row denote the statistically significant difference between values at a level of significance of $p < 0.05$ (based on post-hoc Tukey HSD test)

Comparison of total phenols and antioxidant capacity of celery root powder dried by three different methods clearly indicates that convective drying has the most negative effect on these values. This finding is in line with existing researches of Sorouret *et al.* (2015) and Ramachandraiah & Chin (2017), which confirmed a marked loss of total phenols in celery samples after the application of convective drying, especially at higher temperatures. Marić *et al.* (2020) reported reduced total phenols content by about 50% after convective drying at 50°C and about 75% after drying at 70°C in comparison to the fresh carrot. In contrast, many authors have shown that the lyophilization process is successful in preserving total phenols compared to the convective drying (Marić *et al.*, 2020; Kręcisz *et al.*, 2023). The results in this research revealed that celery root powder that underwent osmotic dehydration in molasses followed by lyophilization, exhibited an 8.5% greater total phenols, compared to the lyophilized celery powder alone. Considering that molasses is widely known for its high pnenolic content (Cvetković *et al.*, 2019; Filipović *et al.*, 2024), the highest value of total phenols in the combined dried sample is obviously the contribution of molasses that diffused during the osmotic treatment into the celery root tissue. Also, in terms of antioxidant capacity determined by two different method, celery root subjected to osmotic treatment prior to lyophilization showed the superior values (DPPH 163.28 μmol TE/100 g d.m. and ABTS 577.28 μmol TE/100 g d.m) in comparison with celery root dried using more conventional convective and lyophilization methods. In relation to lyophilization, which was assessed as a highly effecient approach for retaining the antioxidative potential of dried material (Marić *et al.*, 2020), convectively dried celery root had about 70% lower values of antioxidant capacity assesed by both employed methods (Table 1). In consistency with the current results, Kręcisz *et al.* (2023) reported that the drying method significantly affected the total phenols content and antioxidant capacity of celery, with lyophilization resulting in higher values compared to convective drying. Compared to the lyophilized sample, celery root processed by combined drying showed improved antioxidant capacity - 15.6% higher by the DPPH assay and 2.5% higher by the ABTS test, due to the molasses input, which is recognized for its pronounced antioxidant potential (Chen *et al.*, 2017; Mordenti *et al.*, 2021).

Regarding the influence of the drying type on the brightness and the share of yellow tone of celery root, the lyophilization proved to be the most effective. Convective drying showed the most negative influence on the brightness of the celery sample. The results revealed that convectively dried celery is even darker than the combinedly dehydrated, with higher share of red and yellow tone. Unlike convectively dried celery root, where the dark color comes from enzymatic and non-enzymatic browning reactions in the presence of oxygen at an elevated temperature (50°C) (Kręcisz *et al.*, 2023), the darkness of the combined dried sample comes from molasses that penetrated into the celery tissue during osmotic treatment. Sugar beet molasses owes its deep coloration mainly to melanoidins and caramelization substances formed during the industrial sucrose production (Šobot *et al.*, 2019).

Based on sensory assessment, the intensity of the celery root sample coloration is most pronounced after combined drying, followed by the convectively dried sample. After the lyophilization procedure, sample retained the taste and odour characteristic for celery root. The most marked variation from the typical flavor and scent of celery root (grade 4) was characterized for the convectively dried powder (grade 5.5 and 5.9), while the combined drying caused less pronounced deviation (grade 3.2 and 3.0). A lower deviation from the standard taste and odor after combined drying can also be rated as desirable because molasses masks the pronounced herbaceous and punget aroma of celery root.

PCA was applied to discover the correlation structure between experimentally determined responses of antioxidant characteristic, instrumental colour and descriptive sensory analysis and applied different types of drying. The scatter plot of the PCA (Figure 1) conducted on the tested samples, it provides additional insights and a visual representation of data trends and their ability to distinguish groups (Filipović *et al.*, 2024). The first two principal components (represented at x-axis and y-axis) of the data matrix high accounted of the total variance in the experimental dataset (for 100%).

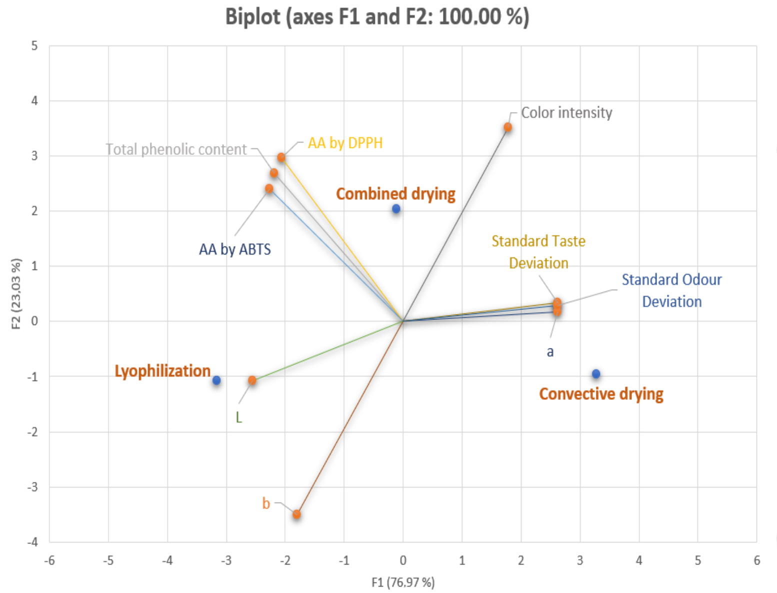

Figure 1. PCA scatter plot diagram depiciting the relationships among selected parameters of celery root samples reagrading dehydration type

A noticeable separation of the three tested celery root samples was achieved, because of the distinct differences in quality responses, as evident from the presented scatter plot diagram. The position of celery root samples on the scatter plot was predominantly determinated by dehydration type, where the combinedly dried and liophilized samples were positioned on the left side of the biplot (negative values of the first principle component (F1)), and convectively drying sample was arranged on the right side of diagram, characterized by positive values of F1. The responses' projection suggested that total phenols content and anioxidant capacities measured by DPPH and ABTS were located at higher positive values of the second principal component. The difference between antioxidant activities and phenols content for combined dried and liophilized samples were less profound, since both samples shared similar responses patterns, signifying the positive effect of these two drying type on these nutritive quality properties. The most pronounced L and b values were identified for liophilized sample and were positioned at the bottom left part of the biplot. Convectively dried sample was characterized by high standard taste and odour deviation and a value. The color intensity was distributed between combinedly and convectively dried sample, showing a slightly more positive correlation with combined drying. All tested responses except color intensity and b value exerted a significant contribution to the first principal component.

CONCLUSIONS

Based on the results, osmotic-pretreatman in molasses in combination with lyophilization, influenced higher values of the total phenols content and antioxidant capacity of the examined celery root than lyophilization, which is considered a very successful method in preserving antioxidant capacity. Also, it was shown that combined drying had a less pronounced influence on the deviation from the color and sensory parameters of celery root, compared to convective drying.

It can be concluded that combined drying, in addition to providing an economic advantage, energy efficiency and an ecological advantage due to the inclusion of by-products in the process, also affects the improvement of the antioxidant potential of the dried material. Future research should be focused on the possible application of combined dried celery powder enriched with bioactive components from molasses as an ingredient of various food products.

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UTICAJ RAZLIČITIH METODA DEHIDRACIJE NA ODABRANE PARAMETRE KVALITETA KORENA CELERA

Sažetak

U ovom radu ispitan je uticaj različitih metoda dehidratacije (konvektivno sušenje, liofilizacija i kombinovana osmodehidratacija i liofilizacija) na parametre boje, senzorne atribute i antioksidativna svojstva korena celera. Uzorci korena celera su sušeni konvektivno u sušari na 50°C do konstantne mase, u liofilizatoru 48 sati, i kombinovanom dehidratacijom koja je uključivala niskoenergetski osmotski predtretman u melasi šećerne repe i skraćenu uzastopnu liofilizaciju u trajanju od 24 sata. Potom su dehidrirani uzorci usitnjeni u prah i podvrgnuti instrumentalnoj analizi boje (L, a, b koordinate), senzornoj analizi (intenzitet boje, ukus i miris) i analizi ukupnog sadržaja fenola i antioksidativne aktivnosti (određeno ABTS i DPPH metodama). Rezultati su pokazali da je konvektivno osušeni celer tamniji od kombinovano dehidriranog, sa većim učešćem crvenog i žutog tona. Najizraženije odstupanje od standardnog ukusa i mirisa (ocena 4) je okarakterisano za konvektivno sušeni prah (ocene 5,5 i 5,9), a potom kombinovano dehidrirani celer (ocena 3,2 i 3,0). Što se tiče uticaja tipa dehidratacije na antioksidativni potencijal, najefikasnijim se pokazala kombinovana metoda, DPPH 163,28 mmol TE/100 g d.m. i ABTS 577,28 mmol TE/100 g d.m, zbog sadržaja melase (odličan izvor antioksidanata) u dehidriranom korenu celera. Prah korena celera koji je prethodno osmodehidriran u melasi imao je čak 8,5% veći sadržaj ukupnih fenola u odnosu na liofilizovani prah, iako se liofilizacija smatra jednom od najboljih metoda dehidratacije u smislu očuvanja fenolnih jedinjenja.

Ključne reči: *kombinovana dehidratacija, liofilizacija, osmodehidratacija, koren celera, antioksidativna aktivnost*

PRODUCTION OF BREADSTICKS ENRICHED WITH HAWTHORN FRUIT*

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Original scientific article

Abstract

The common hawthorn (*Crataegus monogyna*), is a member of the Rosaceae family that is native to Europe, Africa, and Asia. Scientific research has shown that the presence of various bioactive substances such as epicatechin and chlorogenic acid in hawthorn fruit results in strong antioxidant and free radical scavenging properties. This study investigated the preparation of hawthorn fruit-enriched breadsticks. The study aimed to produce a healthy snack with health-promoting effects and evaluate its nutritious and antioxidant properties. Breadsticks were prepared by adding different ratios of hawthorn to the dough (5%, 10%, 15%). Proximate (moisture, ash, fat, protein), total phenolic content and sensory analyses were performed. Hawtorn-enriched breadsticks were compared with no hawthorn-added breadsticks. The sensory analysis showed only a color difference between the hawthorn containing different amounts of breadsticks. According to the evaluation of the participants, there was no statistically significant difference in odor, brittleness, flavor and general liking among the breadstick samples ($p>0.05$). According to the results, as the amount of hawthorn powder increased, the amount of total phenolic substance in the samples increased at a statistically significant level ($p<0.05$). This shows that the aim of enriching the phenolic content with adding hawthorn was successfully achieved. Further studies will focus on optimizing the recipe to show better sensory qualifications.

Keywords: *Hawthorn, breadstick, total phenolic content, sensory analysis, food enrichment*

INTRODUCTION

Adequate and balanced nutrition strengthens the immune system, prevents non-communicable diseases, and indirectly extends human life (Bojang and Manchana, 2023). On the other hand, malnutrition or nutrient deficiencies still remain an important risk affecting human health.

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Micronutrient deficiency is a global public health problem and can cause severe diseases. Iron, folate, vitamin A, zinc, and iodine are generally the most common micronutrient deficiencies (Demircioğlu and Aslan, 2023). The vitamin-mineral content of foods varies depending on various factors such as soil and irrigation conditions, food preparation, and cooking methods (Uluğ and Rakıcıoğlu, 2019). Food fortification techniques have gained significant attention in recent years as a solution to the problem of how to acquire these vitamins and minerals, which are often lost. Enriched foods are useful when micronutrients are either insufficient or impossible to obtain from the food (Demircioğlu and Aslan, 2023).

Since it was determined that the antioxidant properties of wild fruits growing spontaneously in nature are higher than cultivated fruits, the demand for wild fruits has increased in recent years (Tüysüz *et al*., 2021). Fruits such as hawthorn, pomegranate, elderberry, blackcurrant, and raspberry are wild fruits (İlhan et al., 2019). Wild fruits seem a good option for a food fortification approach since they grow spontaneously without human influence and contain many minerals and vitamins that are essential for maintaining a healthy life (İlhan *et al*., 2019; Şengün and Yücel, 2015). They provide health-promoting effects thanks to not being exposed to chemical applications; their composition is rich in antioxidants and composed entirely of natural compounds (İlhan *et al*., 2019).
Hawthorn stands as a good candidate for new and healthier recipes. Numerous research have demonstrated the anti-inflammatory, anti-cancer, antioxidant, cardioprotective and digestive-enhancing qualities of hawthorn. Its primary chemical constituents, pentacyclic triterpenoids (ursolic acid, hawthornic acid, and oleanolic acid), and polyphenols (chlorogenic acid, proanthocyanidin B2, epicatechin, proanthocyanidins, mucoxanthin, quercetin, and rutin) are linked to its bioactive effects (Zhang *et al*., 2022). Hawthorn was determined as a "Traditional Herbal Medicinal Product" by the European Medicines Agency Herbal Medicinal Products Committee. It is also included in the pharmacopoeias of countries such as China, Germany, France, and England (Alkan and Gülmemiş, 2022). It is used in the treatment of diabetes in Mexico; in the treatment of cardiovascular diseases, digestive disorders, hyperlipidemia, and hypertension in China and Korea; in the treatment of diabetes, cardiovascular diseases, and cancer in traditional Arab medicine; and in the treatment of hemorrhoids, sore throat, cough, nephritis and rheumatism in Türkiye. It is consumed as raw fruit or used to produce vinegar. In addition, hawthorn is used in producing sunscreen in cosmetics, as a coating agent in industry, and in preparing punch and canning thanks to its high pectin content (Güler, 2023). This study aimed to produce hawthorn fruit-enriched breadsticks with health-promoting effects thanks to the high antioxidative potential of hawthorn.

MATERIALS AND METHODS

Preparation of hawthorn powder

The hawthorn was picked from Elazığ, Çöteli district in Türkiye. The supplied hawthorns were washed to remove dirt then the seeds were removed. The hawthorns were put through a grinder. Ground hawthorn was dried in the oven at 50ºC, so that hawthorn powder (HP) was produced (Figure 1) and stored at -18 ºC until use.

Figure 1. The hawthorn powder used in the study

Preparation of breadsticks

The increasing amounts of HP (5%, 10%, and 15%) were used to replace the wheat flour in breadstick formulations given in Table 1. Fresh yeast was mixed with warm water (37°C) and waited 10 min to activate. Later, all ingredients were mixed together for 10 min. The prepared breadsticks were fermented for 30 minutes at 40°C and then divided into 80 g pieces. Then, the shaped breadsticks with hawthorn were baked in the oven at 150ºC for 50 minutes. Finally, they were left to cool. After cooling, breadsticks were weighed. The loss as a result of baking was calculated and baking loss was determined.

Table 1. Formulations of the breadsticks produced in the study.

Ingredient	0 HP	5 HP	10 HP	15 HP
HP (%)	%0	%5	%10	%15
HP (g)	-	30	60	90
Wheat Flour (g)	600	570	540	510
Sugar (g)	24	24	24	24
Salt (g)	9	9	9	9

Yeast (g)	15	15	15	15
Oil (ml)	125	125	125	125
Water (ml)	240	240	240	240

* HP; hawthorn powder

Proximate analysis

The dry matter amount was determined according to the AOAC 930.04 standard method. 2 g of samples were dried at 105°C for 3 hours using a drying oven (Memmert, Germany). The total ash amount of the samples was determined according to AOAC 923.03 standard method. 2 g of each sample was burned at 550 °C using a muffle furnace (Carbolite Gero, England) (AOAC, 1995).

Total fat content was determined using TSE EN ISO 11085 method (TSE, 2016). Protein determination was made using the Bradford method, which is applicable to cereals (Morosan *et al*., 2022).

Total phenolic content determination

Total phenolic contents (TPC) of the samples were measured according to Zlatanovic *et al*. (2019) by using Folin Ciocalteu method. For analysis, breadsticks were ground using a coffee grinder (Bosch, Germany). Ground breadsticks (100 mg) were extracted with 1400 µL mixture of ethanol and water (1:1) at room temperature for 60 min. Supernatant was obtained by centrifugation at 12,000 rpm for 10 min. After extraction, the extracts (0.25 ml) were mixed with 1.25 ml Folin-Ciocalteu's phenol reagent (10-fold dilution) and left 6 min for reaction. Later, 1 ml sodium carbonate solution (75 g/l,) was added into the mixture and shaken. After reacting for 2 h at room temperature in dark, absorbance was measured at 765 nm using a spectrometer (UV 1800 Shimadzu, Japan). The results were calculated using a calibration curve (R^2=0,99) and expressed as mg of gallic acid equivalents per gram of sample (mg (GAE)/g).

Sensory analysis

The sensory attributes of the breadsticks were performed with 20 untrained panellists consisting the staff of Faculty of Health Sciences in Bayburt University. The breadstick sensory analysis was performed using a 7-point hedonic scale (1= dislike; 2 = dislike moderately; 3 = dislike slightly; 4 = neither like nor dislike; 5 = like slightly; 6 = like moderately; and 7 = like very much) (Altuğ Onoğur and Elmacı, 2011). The fresh breadstick samples were placed onto the identical plate and coded with different 3-digit numerical codes. Each sample was presented to the panelists in a randomized order so that each sample appeared in a particular position. The taste-free water was used for palate cleansing between samples. Then, they were asked to rate the samples based on a scale for each of the following attributes: colour, odor, brittleness, flavour, and general liking.

Statistical analysis
Analyses were performed in triplicate. Data were compared by ANOVA, including post hoc comparison Tukey's test, at the probability level $\alpha = 0.05$ using IBM SPSS Version 22 for Windows.

RESULTS AND DISCUSSION

HP addition showed an effect on the moisture of the breadsticks, and the control breadstick (0% HP added) had the highest moisture (Table 2). On the other hand, there was no significant different between HP added breadsticks. 15% HP added breadsticks had the highest ash content, possibly thanks to high mineral content of hawthorn as reported in a previous study (Borczak *et al.,* 2016). Although four formulations had the same amount of oil in the recipes, HP addition significantly increased fat content. Protein determination results showed that using hawthorn instead of wheat flour in the formulation decreased the breadsticks' protein content (Table 2). The protein content of 0% and 5% HP-added breadsticks were at the same level and were found to be significantly higher than 10%, and 15% HP-added breadsticks ($p<0.05$). This result can be explained by hawthorn's lower protein content than wheat flour. Fresh hawthorn was reported to have a protein content of %3,03 (Zhang *et al*., 2022). There was no significant difference between samples in terms of baking loss.

Table 2. Compositions and the baking loss of the breadsticks

Samples	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Baking loss (%)
0 HP	$5{,}07\pm0{,}99^{a}$	$2{,}29\pm0{,}21^{ab}$	$14{,}58\pm0{,}11^{d}$	$9{,}02\pm0{,}16^{a}$	$28{,}75\pm4{,}33^{a}$
5 HP	$2{,}90\pm0{,}20^{b}$	$1{,}97\pm0{,}23^{b}$	$15{,}38\pm0{,}08^{c}$	$9{,}64\pm0{,}45^{a}$	$30{,}41\pm0{,}72^{a}$
10 HP	$2{,}43\pm0{,}10^{b}$	$2{,}46\pm0{,}17^{ab}$	$16{,}13\pm0{,}17^{b}$	$7{,}59\pm0{,}33^{b}$	$30{,}83\pm0{,}72^{a}$
15 HP	$2{,}71\pm0{,}3^{b}$	$2{,}57\pm0{,}19^{a}$	$17{,}04\pm0{,}01^{a}$	$5{,}28\pm0{,}00^{c}$	$33{,}33\pm0{,}72^{a}$

* HP; hawthorn powder. Different superscript letters indicate a significant difference between samples (Tukey,$p<0.05$). The results are expressed as mean ± SD.

According to the results, as the amount of hawthorn increased, the amount of total phenolic content in breadsticks increased at a statistically significant level ($p<0.05$). This shows that the aim of enriching the phenolic content by adding hawthorn was successfully achieved, as seen in Table 3. A previous study investigating the effect of wild fruits on the nutritional properties of wheat flour bread also included hawthorn in the study design. The results revealed that bread with 5% lyophilized hawthorn had significantly higher content of total phenolic content and antioxidant activity compared with the control bread ($p < 0.05$) (Borczak *et al*., 2016).

Table 3. Total phenolic content of the breadsticks

Samples	Total phenolic content (mg GAE/ g sample)
0 HP	$0,67 \pm 0,01^{d}$
5 HP	$0,98 \pm 0,04^{c}$
10 HP	$1,06 \pm 0,00^{b}$
15 HP	$1,17 \pm 0,04^{a}$

* HP; hawthorn powder. Different superscript letters indicate a significant difference between samples (Tukey, $p<0.05$). The results are expressed as mean ± SD.

The sensory analysis revealed that as the hawthorn percentage increased, there was no change in sensory properties other than color. Panelists did not like the color change due to the increased amount of hawthorn fruit, as seen in Figure 2. According to the evaluation of the participants, there was no statistically significant difference in odor, brittleness, flavor, and general liking among the breadstick samples ($p>0.05$); the results are given in Table 4.

Figure 2. Breadsticks produced in this study. HP; hawthorn powder

Table 4. Sensory properties of the breadsticks

Properties	0 HP	5 HP	10 HP	15 HP
Color	5,75±1,48^{a}	4,50±1,5^{b}	4,70±1.17^{ab}	4,10±1.44^{b}
Odour	5,15±1,22^{a}	4,70±1,62^{a}	4,55±1,39^{a}	4,15±1,34^{a}
Brittleness	4,60±1,75^{a}	4,25±1,74^{a}	4,00±1,62^{a}	4,20±1,24^{a}
Flavor	5,05±1,31^{a}	4,60±1,66^{a}	4,35±1,42^{a}	3,85±1,38^{a}
General liking	5,25±4,90^{a}	4,90±1,58^{a}	4,60±1,14^{a}	4,50±1,14^{a}

* HP; hawthorn powder. Different superscript letters indicate a significant difference between samples (Tukey,$p<0.05$). The results are expressed as mean ± SD.

CONCLUSIONS

This study reported no negative effects of hawthorn addition on the sensory properties of breadsticks except the color. Further studies will focus on optimizing the recipe to show better sensory qualifications. For instance, using freeze-drying may improve the hawthorn powder's color, enhancing the color of breadsticks. Hawthorn powder addition significantly increased total phenolic content of breadsticks and 10% hawthorn powder added breadstick seems to be better compared to others for sensory attributes. Future studies can focus on developing value-added foods or supplements using this wild fruit.

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PROIZVODNJA KRUŠNIH ŠTAPIĆA OBOGAĆENIH PLODOVIMA GLOGA

Sažetak

Obični glog (*Crataegus monogyna*) član je porodice Rosaceae, a porijeklom je iz Europe, Afrike i Azije. Naučna istraživanja pokazala su da prisutnost raznih bioaktivnih tvari poput epikatehina i hlorogene kiseline u plodu gloga rezultira snažnim antioksidativnim svojstvima i svojstvima hvatanja slobodnih radikala. Ova je studija istraživala pripremu krušnih štapića obogaćenih plodom gloga. Cilj studije bio je proizvesti zdravu grickalicu s učincima koji promiču zdravlje i procijeniti njegova nutritivna i antioksidativna svojstva. Krušni štapići pripremljeni su dodavanjem različitih omjera gloga u tijesto (5%, 10%, 15%). Provedene su približne analize (vlaga, pepeo, masti, proteini), ukupnog sadržaja fenola i senzorne analize. Krušni štapići obogaćeni glogom uspoređeni su s krušnim štapićima bez dodanog gloga. Senzorna analiza pokazala je samo razliku u boji između gloga koji sadrži različite količine krušnih štapića. Prema procjeni sudionika, nije bilo statistički značajne razlike u mirisu, krhkosti, okusu i općem ukusu među uzorcima hljeba ($p>0,05$). Prema rezultatima, s povećanjem količine glogovog praha, količina ukupnih fenolnih tvari u uzorcima povećavala se na statistički značajnoj razini ($p<0,05$). To pokazuje da je cilj obogaćivanja fenolnog sadržaja dodavanjem gloga uspješno postignut. Daljnja istraživanja usredotočit će se na optimizaciju recepta kako bi se pokazale bolje senzorne kvalitete.

Ključne riječi: *Glog, hljeb, ukupni fenolni sadržaj, senzorna analiza, obogaćivanje hrane*

THE PERCEPTION ON USAGE OF THE FOOD PRESERVATIVES BY CONSUMERS IN NORTH MACEDONIA*

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Original scientific paper

Abstract

Preservatives are one of the most commonly used additives in many food products, such as: soft drinks, fruit and vegetable products, processed meat, various sweet desserts, mayonnaise, ketchup, sauces, wines and beers, some types of milk products, etc. Their use in our country, as well as in other countries around the world, is regulated by law. As part of this research, an anonymous survey of different population groups (children, adolescents and adults) was conducted in order to determine how familiar consumers are with the term food additives, and especially with the preservatives, the benefits of their use versus the adverse effects on human health. The questionnaire consists of 29 questions, divided into 4 groups, namely Demographic data, Knowledge of healthy habits, Knowledge of additives and Knowledge and use of preservatives. The results of the survey conducted on 392 participants from North Macedonia show that the largest percentage of respondents (67.60%) answered that they know what preservatives are, but when asked to list which preservatives they know, only 26.28% of respondents answered correctly, 5.87% partially correctly, 8.42% incorrectly, and 59.44% did not know how to answer. When asked if they were informed about the risk of consuming large amounts of preservatives in the diet, 50.26% respondents answered affirmatively, 38.01 % were partially informed, and 11.73% were not informed. The results of this research indicate that there is a need for better education and awareness of consumers about the use of preservatives in their diet, as well as about the risks of their intake.

Keywords: *consumer's perception; food preservatives; survey*

INTRODUCTION

Preservatives are chemical substances capable of retarding or arresting the growth of microorganisms to prevent processes such as fermentation, acidification, or decomposition, which cause deterioration of flavor, color, texture, appearance, and nutritive value.

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Preservatives are often used in combination with physical methods; such combinations may allow the preservatives to be used at lower concentrations, thus retaining the quality of the product (Samal *et al*., 2017; Surekha and Reddy, 2014).
The most widely used preservaties added to certain foods are: benzoates (E210 -E219), benzoic acid (E210) or its sodium salt (E211), sorbates (E200 – E209), potassium (E212) or calcium (E213) salt. Sodium benzoate and benzoic acids are mostly used in acidic food products like fruit juice, carbonated drinks, pickles and jams (Samal *et al*., 2017). Soft drinks are the major contributor to the dietary benzoic acid exposure in many Western countries (WHO, 1999). However, there are large differences in the permitted levels for soft drinks among international, national and regional standards (Ming Maa *et al*., 2009). The use of benzoic acid is authorized in the European Union in several food groups on the condition of respecting the maximum use levels (Directive 95/2/EC; European Commission 1995), which are all expressed as benzoic acid equivalents (Vandevijverea *et al*., 2009).
Different amounts are required for effective antimicrobial activity. Benzoic acid inhibit yeast at pH 4. The most common preservatives are benzoates (sodium benzoate), used in the range 0.01 – 0.10 % and sorbates (sorbic and potassium sorbate) used in the range 0.03 – 0.10 % (Garcıa-Garcıa and Searle, 2016).
Several scientific bodies have conducted toxicological evaluations of benzoic acid and its salts and have established the acceptable daily intake (ADI) of 0 to 5 mg/kg body weight per day, expressed as benzoic acid equivalents (Darch *et al*., 2021).
Although preservatives are beneficial for packaged food, they do have some adverse effect on human health. For example, the cytotoxicity of sodium benzoate, as a food preservative, have been demonstrated. All preservatives cause hyper activity on regular usage (Kane *et al*., 2024; Samal *et al*., 2017). Sodium benzoate has also been linked to hyperactivity in children when mixed with other artificial food colors (McCann *et al*., 2007). Benzoates are strictly forbidden for asthma patients because it worsens the condition. Benzoates are also reported to cause rhinitis, chronic urticarial and flushing in some cases. Sodium benzoate is found to form carcinogenic benzene while used with vitamin C or ascorbic acid. Though the amount of benzene form is low but it is a risk factor to cause cancer. It is also reported that benzoates can cause brain damage. Sorbates can cause urticarial and contact dermatitis in some cases (Samal et al., 2017).
The main aim of this research was to determine how familiar are the consumers in North Macedonia with the term food additives, especially the preservatives and the benefits of their use versus the adverse effects on human health.

MATERIALS AND METHODS

This research was conducted through an anonymous survey involving 392 participants from North Macedonia over the age of 12. The average time to complete the survey was about 13 minutes. This anonymous online survey was conducted in the period from February to June 2022 on the territory of the Republic of North Macedonia.

The questionnaire was prepared by the authors of the paper and consisted of 29 questions, divided into four groups: Demographic data (5 questions), Knowledge of healthy habits (11 questions), Knowledge of additives (5 questions) and Knowledge and use of preservatives (8 questions). The questionnaire was distributed electronically through the media platform Forms, to professors, colleagues, students, as well as their friends and relatives. After completing the research period, a statistical analysis of the collected data was performed by using Microsoft Excel. The first step consisted of a tabular presentation of the responses received to each individual question, followed by summarizing the data. After statistical processing, the results are presented in analytical and graphic form.

RESULTS AND DISCUSSION

Demographic data were presented in the first part of the survey, consisting of 5 questions, where a sociodemographic characterization was made in order to obtain a more detailed description of the participants. Namely, the questions were aimed to determine the age, gender, living environment, level of completed or current education and the occupation or profession of the participants. According to the obtained results, most of the participants 269 (68.62%) were aged 30 – 64 years; 90 (22.98%) participants were aged 19 – 29; 28 (7.14%) were aged 12 – 18; and 5 (1.30%) were older than 64 years. Of the 392 participants, 325 (82.90%) were female, while 67 (17.10%) were male. Of them, 366 (93.37%) lived in cities, and 26 (6.63%) lived in rural areas. From the obtained responses regarding the degree of completed or current education, it can be concluded that the majority of respondents, 203 (51.80%) had BSc degree, 74 (18.90%) had completed secondary education, and 35 (8.90%) were students. This is followed by 50 participants (12.80%) who had completed master's studies, while 26 respondents (6.60%) had completed doctoral studies. 2 respondents (0.50%) had completed specialization, 1 participant (0.25%) had primary education, and 1 participant (0.25%) answered that is not relevant. To the question about profession, most of the respondents were: graduated engineers, doctors, pharmacists (78; 19.90%), graduated economists, lawyers (73; 18.62%), students (41; 10.46%), academic professors (36; 9.18%), teachers (30; 7.65%), technicians and laboratory workers (secondary education), (24; 6.12%), economists/lawyers (secondary education), (22; 5.61%); pupils (7; 1.78%). Out of the total number of respondents, 12 (3.06%) were unemployed, while 69 (17.60%) answered that they had "other" occupation. *Knowledge of healthy habits* were presented in the second part of the survey, which contained 11 questions in order to determine how much the participants know and practice healthy habits. The first three questions refer to the body mass and height of the participants. According to their answers, the majority of respondents, 151 (38.52%) had a body mass in the range of 60 to 70 kg, 83 (21.17%) had more than 80 kg, 80 (20.41%) had less than 60 kg, while 78 (19.90%) respondents had a body mass in the range of 71 to 80 kg.

In terms of body height, the responses of the participants were as follows: 222 (56.63%) had a body height between 160 and 170 cm, 111 (28.3 %) participants were between 171 and 180 cm tall, 31 (7.91%) had a body height above 180 cm, while 28 (7.14%) were below 160 cm. The BMI is calculated by dividing an adult's weight in kilograms by their height in meters squared. Regarding the respondents' opinion about their body mass, 224 (62.24%) thought that it was within normal limits, 138 (35.21%) participants were overweight, and 10 (2.55%) of them thought that they had a lower body mass than normal. A higher BMI increases the risk of developing long-term conditions, such as type 2 diabetes and heart disease.

The next few questions refer to whether the respondents played sports, to which the majority, 189 (48.22%) answered that they played sports recreationally, also a large number of them, 169 (43.11%) answered that they did not play sport at all, while 34 (8.67%) actively played sports. Regarding the type of sport, most of them did: walking 51 (32.08 %), running 16 (10.06 %), fitness/gym 20 (12.58%), cycling 13 (8.18%), football/basketball 10 (6.29%), volleyball /handball 6 (3.77%), yoga/pilates 14 (8.80 %), martial arts 14 (8.80%), and 15 (9.44%) played other sports.

Furthermore, participants were asked, if they slept at night, where 354 (90.30%) responded that they did, 23 (5.87%) sometimes slept, and 15 (3.83%) did not sleep. In addition to this question, for those who did not sleep, followed the question "If not, is it because of work responsibilities or personal choice", where 73.33% did not sleep due to work obligations, and 26, 67% did not sleep due to personal choice. Of the participants who answered "Sometimes", 50.00 % did not sleep due to work obligations, and 50% did not sleep due to personal choice.

Then followed questions in order to determine whether the participants pay attention on the quality of their diet. Thus, to the question "Do you pay attention to the quality of your diet?", the majority of respondents 224 (57.14%) answered affirmatively, 144 (36.74%) answered that they sometimes paid attention, and 24 (6.12%) did not pay attention to the quality of the diet. To the question "Do you pay attention to the caloric value of your diet?", 152 (38.78%) respondents answered that they did not pay attention, approximately as many respondents (146, 37.24%) answered that they sometimes paid attention, while 94 (23.98%) respondents answered affirmatively.

In addition, the participants were asked whether they were informed about the positive effects of the products they consume, where 218 (55.61%) answered affirmatively, 136 (34.70 %) responded that they were partially informed, and 38 (9.69%) were not informed. Similar results were also obtained when asked whether they were informed about the negative effects of the products they consumed, where 230 (58.67%) responded affirmatively, 125 (31.89%) responded "partially", while 37 (9.44%) responded "no".

The third part of the survey consisted of 5 questions on *Knowledge of additives*. The questions and the obtained results are presented in Figure 1.

For the question "Do you know what food additives are?" the majority of respondents, 295 (75.26%) knew what food additives are, 82 (20.92%) had partial knowledge, while 15 (3.82%) did not know what food additives are. As addition of the previous question, followed "Do you think you are sufficiently informed about the meaning of food additives?", where the majority of respondents, 162 (41.33%) answered that they were partially informed, 141 (35.97%) answered that they were sufficiently informed, and 89 (22.70%) answered that they were not sufficiently informed about the meaning of food additives.

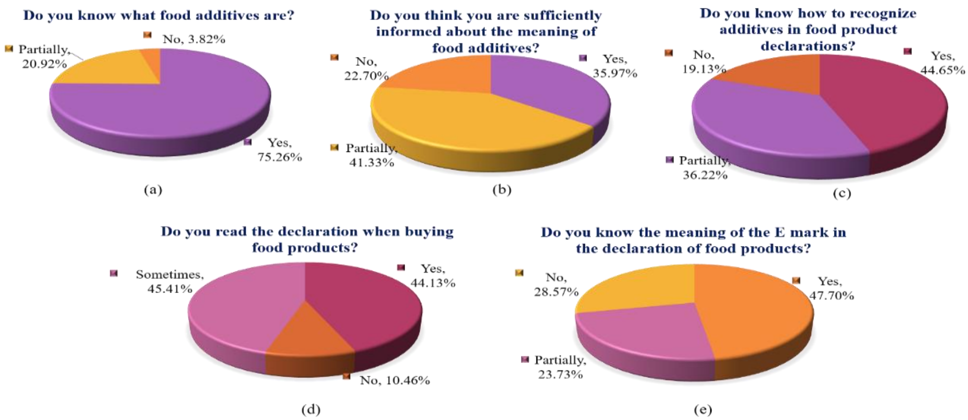

Figure 1. The answers to the questions about the knowledge of additives

When respondents were asked "Do you know how to recognize additives in food product declarations?" less than half, 175 (44.65%) respondents knew how to recognize additives, 142 (36.22%) partly knew, while 75 (19.13%) did not know. Then, the respondents were asked if they read the composition of the declaration when buying food products, the majority of respondents 178 (45.41%) answered that they sometimes read it, approximately the same number of respondents, 173 (44.13%) answered affirmatively, while 41 (10.46%) respondents answered that they did not read it.

The next question in the survey was whether did consumers know the meaning of the E number in the declaration of food products. The majority of respondents, 187 (47.70%) knew it, but this number was less than half of the respondents. Of those surveyed, 93 (23.73%) answered that they partially knew it, and 112 (28.57%) did not know the meaning of the E number in the food product declaration.

Knowledge and use of preservatives, given in the fourth part of the survey was in purpose to obtain more detailed information about how much did the respondents know and use preservatives. As can be seen in Figure 2, the first question from this section was "Do you know what preservatives are?", to which more than half of the respondents (265, 67.60%) answered affirmatively, 114 (29.08%) partially knew, and 13 (3.32%) did not know what preservatives are. Then the respondents were asked if they could list some preservatives, to which only 103 (26.28%) participants answered correctly, and 23 (5.87%) answered partially correctly.

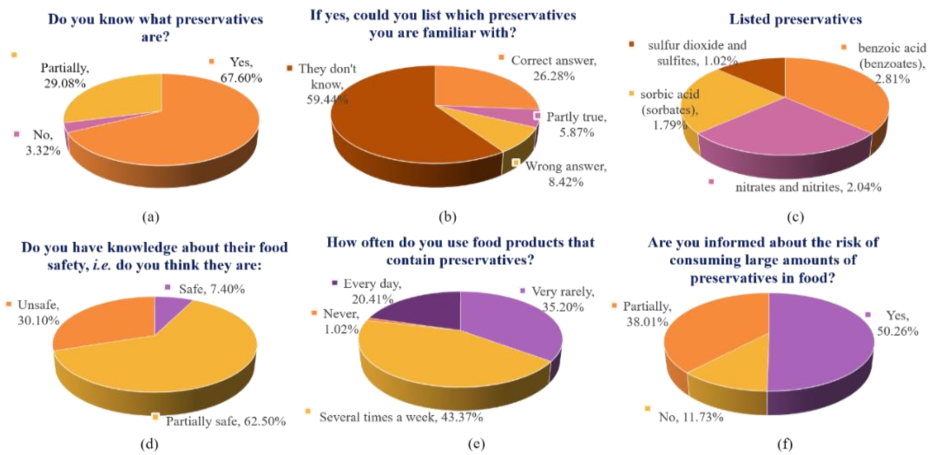

Figure 2. Some answers regarding to knowledge and use of preservatives

According to the answers, more than half of the respondents 233 (59.44%) did not know any preservative, and 33 (8.42%) gave incorect names of substances as preservatives. Of the correct answers, the most listed were: benzoic acid and benzoates (11, 2.81%), nitrates and nitrites (8, 2.04%), sorbic acid and sorbates (7, 1.79%), and sulfur dioxide and sulfites (4, 1.02%). Considering that the use of preservatives can have adverse effects on consumer health, the respondents were asked "Do you have knowledge about their food safety, i.e. do you think they are: safe, partially safe or unsafe?". The obtained answers show that the majority of respondents 245 (62.50%) considered them partially safe, 29 (7.40%) thought that they are safe, while 118 (30.10%) thought that they are not safe.

To estimate the intake quantity of food products containing preservatives, the participants were asked the question "How often do you use food products containing preservatives?". The answers showed that the majority of respondents used products with preservatives several times a week (170, 43.37%), and 138 (35.20%) used them very rarely. Also, 80 (20.41%) participants consumed such products daily, and 4 (1.02%) answered that they never consumed such products. To the question "Are you informed about the risk of consuming large amounts of preservatives in food?", about half of the respondents 197 (50.26%) answered affirmatively, 149 (38.01%) respondents were partially informed, while 46 (11.73%) gave a negative answer.

According to a study by Legesse *et al*. (2016) conducted in Ethiopia, 64% of respondents were unaware of the possible negative consequences of food additives and 70% would continue to consume even after seeing the consequences. At the same time, a large part of consumers were not interested in reading product declarations.

Today, on the market, there is a wide and diverse range of food products containing preservatives. Therefore, the question "Which food products containing preservatives do you use most often in your diet?" was asked with the possibility of multiple answers (Figure 3). Of the products offered, the answers showed that consumers mostly consumed dairy products (275, 70.15%), followed by mayonnaise (167, 42.60%), mushrooms (156, 39.80%) and processed fruit and vegetables (154, 39.28%), then soft drinks (103, 26.27%), wine (74, 18.88%), beverages with a low alcohol content (37, 9.44%), and 70 (17.86%) of responses were given for other products. Additionally, consumers were asked to indicate the daily/weekly consumption amount of food products containing preservatives. According to the obtained results, the majority of respondents (216, 55.10%) did not answer, 36 (9.18%) of respondents did consume very few of them or very rarely, 31 (7.91%) consumed up to 500 g (500 mL) per week, 14 (3.57%) respondents consumed more than 1 kg (1 L) per week, 15 (3.83%) respondents consumed large quantities and very often, 13 (3.38%) respondents consumed often (several times a week), and 62 (15.82%) respondents answered with “other”.

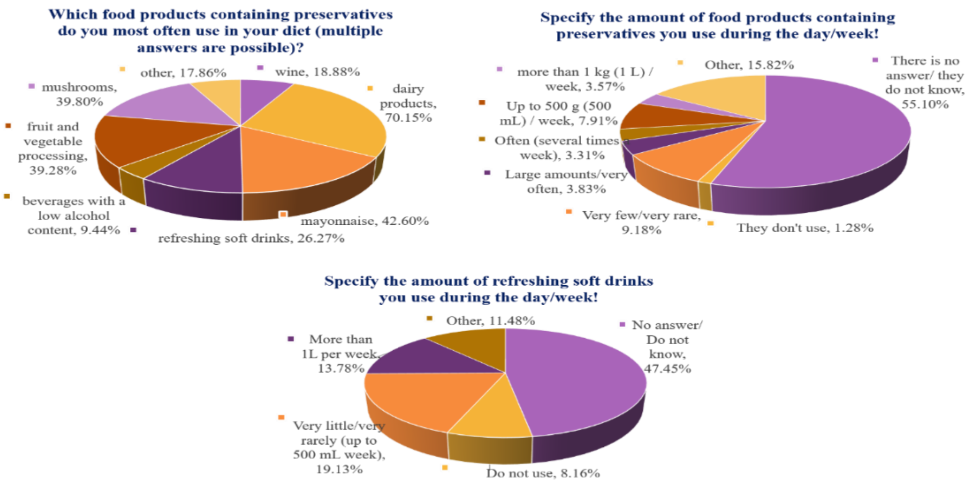

Figure 3. Some answers regarding to knowledge and use of preservatives

As the last question in the survey, respondents were asked to indicate the daily/weekly amount of soft drinks they consumed, where 186 (47.45%) participants did not answer, 32 (8.16%) did not use, 75 (19.13%) used very little / very rarely (up to 500 mL week), 54 (13.78%) had used more than 1L per week and 45 (11.48%) answered "other".

CONCLUSIONS

This research provides an overview of consumer knowledge and use of preservatives by different population groups in the Republic of North Macedonia.
The obtained results showed that consumers are partially informed about the meaning of food additives, very few consume them in large quantities, and most consumers avoid them or use them rarely and in small quantities.

The results of the survey showed that the majority of respondents (67.60%) thought they knew what preservatives are, but 59.44% of them did not know how to specify a single preservative, 8.42% answered incorrectly, 5.87% answered partially correctly, and only 26.28% answered correctly.
The results of this research indicated that there is a need for better education and of raising the awareness of consumers about the use of preservatives in their diet, as well as about the risks of their intake.
These results could be a good base for further research in order to estimate the risk of consuming high amounts of food products containing preservatives.

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PERCEPCIJA UPORABE KONZERVANSA U HRANI OD STRANE POTROŠAČA U SJEVERNOJ MAKEDONIJI

Sažetak

Konzervansi su jedni od najčešće korištenih aditiva u mnogim prehrambenim proizvodima, kao što su: bezalkoholna pića, proizvodi od voća i povrća, mesne prerađevine, raznih deserata, majoneza, kečapa, umaci, vina i piva, neke vrste mlečnih proizvoda itd. Njihova upotreba u našoj zemlji, kao iu drugim zemljama širom svijeta, regulisana je zakonom. U okviru ovog istraživanja sprovedena je anonimna anketa kod različitih populaciskih grupa (djeca, adolescenti i odrasli) kako bi se utvrdilo koliko su potrošači upoznati s pojmom aditiva u hrani, a posebno s konzervansima, prednostima njihove upotrebe u odnosu na štetnog uticaja na ljudsko zdravlje. Upitnik se sastoji od 29 pitanja, podijeljenih u 4 grupe, i to Demografske podatke, Spoznanje zdravih navika, Spoznanje aditiva i Spoznanje i upotreba konzervansa.

Rezultati ankete sprovedene na 392 učesnika iz Severne Makedonije pokazuju da je najveći procenat ispitanika (67,60%) odgovorio da zna šta su konzervansi, ali na pitanje da navedu koje konzervanse znaju, samo 26,28% ispitanika je tačno odgovorilo, 5,87% delimično tačno, 8,42% netačno, a 59,44% nije znalo da odgovori. Na pitanje da li su informisani o riziku konzumiranja velikih količina konzervansa u ishrani, 50,26% ispitanika je odgovorilo potvrdno, 38,01% je delimično informisano, a 11,73% nije informisano. Rezultati ovog istraživanja ukazuju da postoji potreba za boljom edukacijom i podizanjem svijesti potrošača o upotrebi konzervansa u ishrani, kao i o rizicima njihovog unosa.

Ključne riječi: *percepcija potrošača, konzervansi u hrani, anketa*

DIET AS A SIGNIFICANT SEGMENT IN THE SUCCESS OF A TRIATHLON ATHLETE PARTICIPATING IN THE PROJECT: HUMANITY AS A FORGOTTEN OLYMPIC DISCIPLINE*

Tina Tadić[1], Irzada Taljić[1]

Professional paper

Abstract

This work is part of the project "Humanity as a Forgotten Olympic Discipline" which represents a humanitarian expedition where an athlete will be enduring 13 days of a triathlon starting at the Faculty of Agriculture and Food Sciences, Sarajevo, Bosnia and Herzegovina, and finishing at the top of the Olympus Mountain in Greece.
The primary goal of this project was to collect financial resources for the purpose of treating patients with cancer. Through his actions, the triathlon athlete faced extreme challenges which symbolically represent a battle full of ups and downs which these patients are fighting each day. A triathlon is a sport that requires skill, effort, endurance and motivation. It combines three sports disciplines: running, cycling, and swimming. The athlete was engaged in these disciplines during his venture to the Olympus Mountain. Most important aspect of this humanitarian expedition was the power of will. The will to succeed equals the will to get well. In these extreme circumstances, the athlete had support from his team: project coordinator, doctor of medicine, nutritionist, mountain climbing expert and photographer.
Due to daily exhausting physical activity, the role of nutritionist played a significant part in his endeavor. Hence, the dietary plan was adapted to the conditions of staying in camper vans and facing unexpected challenges.
Used methods are: Anthropometry (height, weight, BMI); BIA; software Program Prehrane 5.0; creating a dietary plan for 11 days including food preferences, energy needs and endurance parameters. Expected outcome refers to meeting the nutritional needs of the athlete with the aim of maintain endurance and energy.

Keywords: *humanity, triathlon, dietary plan, endurance, macronutrients*

INTRODUCTION

According to data stated by the USA triathlon (USA triathlon, 2006), the first triathlon was held on Mission Bay in San Diego on September 25, 1974 and consisted of 6 miles of running, 5 miles of cycling, and 500 yards of swimming (Strock *et al*., 2006).

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Endurance sports are becoming increasingly popular and more people are running half marathons, marathons, ultramarathons, half Ironmans, and even Ironman competitions, lasting anywhere between 2 hours and 17 hours (Saris *et al*., 2003). Preciously, triathlon is multidisciplined activity which comprises three sports of swimming, cycling and running (Burns *et al*., 2003) and competitions last between 1 hour 50 minutes (Olympic distances), and 14 hours (Ironman distances). Independent of the distance, dehydration and carbohydrate depletion (CHO) are the most likely causes of fatigue in triathlons, where gastrointestinal (GI) problems, hyperthermia and hyponatremia are potentially health threatening, especially in events of longer duration (Jeukendrup *et al*., 2005). Fatigue during prolonged exercise is often associated with glycogen depletion (Bergström *et al*., 1967; Hultman, 1967) and reduced blood glucose concentrations (Coyle *et al*., 1986) and, therefore, high pre- exercise muscle and liver glycogen concentrations are believed to be essential for optimal performance (Jeukendrup et al., 2005). In addition, Noakes (Noakes, 2000) suggested that it is unlikely that muscle glycogen depletion “alone” limits prolonged exercise performance. Using a stimulated Ironman triathlon model, Noakes predicted that after 4.5 hours of cycling at an estimated exercise intensity of 71% maximum oxygen consumption (VO_{2max}), an elite male Ironman triathlete would have almost completely depleted his or CHO stores. Interestingly, after the completion of the 180 km cycle, elite triathletes are able to run at the speed of 16 km/h for another 160 minutes, which represents an exercise intensity of >66% VO_{2max} (Jeukendrup *et al*., 2005). Considering the afore mentioned results, this review focuses on the athlete’s dietary needs, while facing physically intense endurance in particular circumstances.

Understanding that the “endurance triathlon”, or Ironman triathlon, is a 3-sport event consisting of a 3.8km swim and a 180 km cycle, followed by a 42.2 km marathon run with a duration longer than 4 hours, it can and be considered as “ultraendurance” (Kreider, 1991; Hawley *et al.,* 1995). Therefore, a more appropriate name for the race may be an “ultraendurance triathlon” (UET) (Laursen *et al*., 2001). Referring to that, the project “Humanity as a forgotten Olympic discipline” could be also considered as a “ultraendurance” triathlon in addition of 13- day long endeavor from Sarajevo (BA) to Olympus mountain (GRE). Due to the mentioned expedition, the athlete’s diet played role in fulfilling his energy needs accomplish the project successfully.

Evaluation of Boston Marathon finishers, dating all way back to 1920s, the data showed that those with higher blood glucose levels postmarathon, from prerace glucose loading and race fueling, tended to perform fair better at finish (Gordon *et al*., 1925). CHO was and still is the primary energy source for endurance athletes including triathletes because of its importance as a fuel for muscle and central nervous system (CNS) functioning during moderate- to high-intensity endurance exercises (Jeukendrup, 2013). The ergogenic effects of exogenous CHO consumption during exercises are related to the sparing of skeletal muscle glycogen, prevention of liver glycogen depletion and the subsequent onset of hypoglycemia and/or facilitating high rates of CHO oxidation to fuel moderate- to higher-intensity exercise (Cermak *et al*., 2013). Recommended CHO intake during endurance exercises is based on event duration (Getzin et al., 2017). For exercise lasting between 1 and 2.5 h, 30 to 60 g of CHO per hour has been found to provide adequate exogenous CHO to spare glycogen (Bartlett *et al*., 2015; Burke *et al*., 2013; Thomas *et al*., 2016). For longer duration exercise/competition (>2.5 to 3 h), consuming amounts of CHO up to about 90 g per hour has been associated with better race times (Pfeiffer *et al*., 2012).

Body composition analysis procedures that are carried out today using different methods, are based on a simple two- component (distinguish the proportion of fat and lean mass in the total body mass), three- component (distinguish the proportion of water, fat and lean mass) and four- component (distinguish the proportion of water, bone minerals, proteins and fat) model (Mišigoj- Duraković *et al*., 2014).

The human body generally consists of lean and fat mass. Lean mass is heterogeneous and extremely metabolically active considering that it includes muscle and bone mass, extracellular water, nervous tissue, various organs and all cells except adipocytes (Willet, 2013). Fat mass is metabolically inactive, however, it plays an important role in hormone metabolism and adiponectin levels. It is divided into essential or tissue and non- essential or storage fat. Essential fat is found in a cell’s membranes and is mostly made up of phospholipids and cholesterol (Mandić, 2007).

The most popular and common methods for assessing body composition are those that are relatively inexpensive and provide relatively reliable results, based on a two-component model of body composition such as that obtained using bioelectrical impedance (BIA). Apart from the mentioned methods, there are other, more technologically advanced methods based on multi- component body composition models. Some of them are nuclear magnetic resonance, computed tomography, dual-energy X- ray absorptiometry (DEXA), ultrasound methods, etc. (Duren *et al*., 2008).

BIA, as already mentioned, is a popular method of body composition analysis. It is a reliable, non- invasive method that is widely available. It works on the principle of passing a weak electric current signal through the body, where the voltage drop between two electrodes is proportional to the volume of water in that area of the body. The resistances of different tissues are standardized from previous laboratory measurements where muscle tissue contains a large proportion of water and therefore serves as an electrical conductor, while fat tissue contains a small proportion of water and therefore acts as a resistor to the flow of electrical signals (Mišigoj- Duraković *et al*., 2014). BIA is a very suitable method for epidemiologic research because of its ease of use, low cost, and minimal effort from the subject (Lee *et al.,* 2008).

MATERIALS AND METHODS

With the aim of establishing an optimal CHO fuel along with other macronutrients, the main focus of the athlete's dietary plan was to provide a suitable amount of it in order to fulfill energy needs for daily physical activity. Although we succeed to meet these requirements, we had to take into consideration the most convenient time interval for nutrient consumption and energy loading.

Practical considerations unique to triathlon competition include the lack of opportunity for fueling during swimming. The cycling portion of the triathlon is the most conducive time for CHO ingestion and provides an opportunity for CHO and fluid intake in preparation for the running part (Getzin *et al*., 2017). The concept of the dietary plan was conducted into 3 main meals and 1 side dish. Hence, the breakfast was separated into 2 meals for the purpose of meeting adequate nutritional requirements. As a result of this dietatry plan, the athlete had an early morning meal which was 2 hours before the 20 km of running, followed by prerace CHO ingestion 60 min or 30 min before the mentioned physical activity. Furthermore, the second meal intended to fulfill breakfast requirements was in the time interval between the running and cycling distances. Moreover, the other meals were consumed after the physical activities for each day have been finished, along with the main focus on the athlete's recovery by an imminent nutrient intake. Nonetheless, the athlete had the opportunity to ingest additional calories from CHO fueling such as sports drinks, protein gels or protein bars during the running, cycling and swimming parts.
Prior to preparing the athlete for the enormous intensity of physical activities he would take on each day during this 13 day long expedition, we conducted several methods. Firstly, we measured the athlete's weight and height, which amounts to 100 kg of weight and 206 cm of height. After that, other anthropometric parameters were taken referring to the calculation of his body mass index (BMI) which resulted 23.6 kg^2. Observing established range of BMI values according to the WHO for adults, where a threshold value of 25.0 kg/m^2 indicates increased body mass, and a value of 30.0 kg/m^2 obesity (WHO, 2000), we concluded that the athlete has a normal ratio of weight and height. Subsequently, we approached to athlete with bioelectrical impedance analysis (BIA) in order to determine the athlete's body composition by using small electrodes.
Except BIA, we used a questionnaire as a method for getting reliable data in order to question athlete's usual dietary habits and to evaluate his mental health status. Considering the inevitable difficulties during pro-longed period of this endeavor, possible complications such as dehydration and CHO depletion, we questioned the athlete's previous food preferences with the aim of getting a comprehensive picture of his nutritional status for the purpose of making the most eligible dietary plan adapted to staying in camper vans and facing unexpected challenges. Although last minute project-plan changes meant that instead of sleeping in camper vans, the team would sleep in the apartments, the pace and everyday changing locations mean that the dietary plan was still applicable.
Furthermore, the dietary plan was conceived through the software Program Prehrane 5.0. Additionally, the main focus was creating a dietary plan for 11 days including food preferences, energy needs and endurance parameters which will contribute to the successful accomplishment of the project. Considering that the athlete has spent last two days of this expedition in the mountion, those were determined as a dietary choice. Moreover, we wanted to obtain a balanced intake of nutrients despite the possible complications such as the impossibility to provide necessary nutrients in particular circumstances.

RESULTS AND DISCUSSION

In summary, this review highlights the importance of nutrition in every segment of a physically active cycle. This implies on the different stages of the training process such as pre-competition, race day and post-competition energy requirements. Precisely, it is important to ingest a proper amount of nutrients in the recovery phase. In order to provide sufficient nutrients to the body with the aim of establishing optimal glycogen reserves, we conducted a dietary plan where we wanted to accomplish adequate CHO fueling due to the resting phase. Based on the mentioned, the athlete's 2nd meal which refers to the lunch meal was consider to be the most meaningful.
The dietary plan was adapted for 11 days of regular meals including 3 main meals and 1 side dish as mentioned previously, where the tremendous effort was put into the accomplishment of the macronutrient balance, along with the main focus of CHO intake in order to provide maximum energy capacity for the endurance distances. After the 11th day of dietary plan, remaining 2 days of the project, were adapted to the climbing of Mount Olympus and staying in tents.
Regarding to the athlete's body composition and energy requirements, we created the dietary plan which contained approximately 4000-4500 kcal per day. As we considered breakfast to be the most important for providing energy, the athlete needed to ingest approximately 1700 kcal, which we devided it into two meals: pre running and post running meal. As mentioned before, we put the focus on CHO ingestion in order to provide sufficient energy from whole grain groceries with the aim of keeping blood sugar in balance.

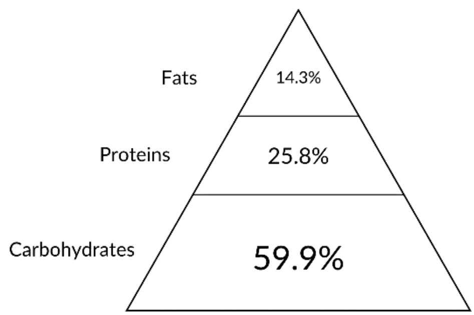

Figure 1. Breakfast consumption

Additionally, after the cycling phase, we wanted to provide the meal as a way to obtain energy and provide propper recovery. We hypothesized the athlete's requirements for fast energy during cycling parts. We provided him with the natural components of fruit such as glucose and fructose, and the second alternative was fast energy-releasing sport drinks, protein gels or protein bars.

The athlete consumed various grains such as rice, sweet potatoes, potatoes, whole grains pasta, etc., in a combination with protein, which was equally presented from animal and plant sources. Lunch meal was recommendated to be consumed after the accomplishment of daily phyisical activity.
Nonetheless the recovery phase, we continued with CHO fueling in the dinner meal as well, since athlete's requrements for energy.

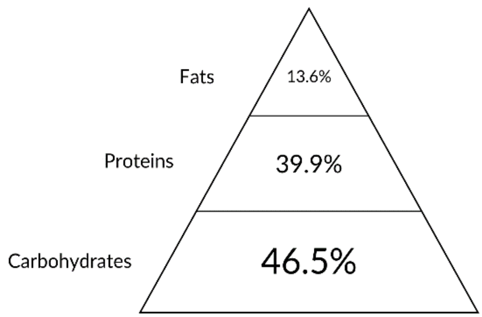

Figure 2. Lunch consumption

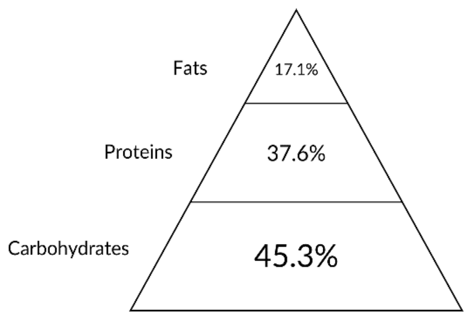

Figure 3. Dinner consumption

It is acknowledged that as reduced blood glucose concentrations (Hultman, 1967) and glycogen depletion (Coyle *et al*., 1986) during endurance exercises have been associated with fatigue, it is important for a triathlete to start such an event with high muscle and liver glycogen reserves (Robins, 2007). Glycogen reserves can be partially depleted following an overnight fast, making it important for the triathlete to replenish these reserves prior to a race. Some athletes do not tolerate CHO and fluid consumption during races, so the prerace meal may be the last opportunity to consume CHO without concern over ill effects during the race, such as gastrointestinal distress. During an Ironman triathlon, endogenous fuel reserves were estimated to supply over half of the energy expended (Kimber *et al*., 2002), providing further support for CHO-loading to maximize endogenous fuel reserves (Robins, 2007). CHO intake will increase blood glucose concentrations and augment CHO oxidation during endurance exercise (Coyle *et al*., 1986). Kimber *et al*. (2002) found that the average CHO intake (94% total energy intake) during an Ironman distance triathlon was 1.0 g/kg BW/h in females and 1.1 g/kg BW/h in males, which was sufficient to support the previously proposed maximum rate of CHO utilization of 1.0 g × kg-1 × h-1 (Hawley *et al*., 1992). Kimber *et al*. (2002) found that this level of CHO consumption (1–1.1 g/kg BW/h) was achieved by consuming large amounts (~1.5 g/kg BW/h) during the cycling phase of the race, approximately three times as high as that consumed during the marathon run. Considering the above mentioned, the athlete had energy requirements for CHO fueling during the running phase instead of usual cycling phase requirements. Additionally, his lower extremities were trembling after the 10 km running distance since he felt fatigue and needed to ingest fast energy releasing source of food.

As a result of our specific dietary plan, we can agree with Thomas *et al*. (2016) who stated that nutrition plans need to be personalized to the individual athlete to take into account the specificity and uniqueness of the event, performance goals, practical challenges, food preferences, and responses to various strategies. Some nutrients (eg, energy, CHO, and protein) should be expressed using guidelines per kg of body mass to allow recommendations to be scaled to the large range in the body sizes of athletes. Sports nutrition guidelines should also consider the importance of the timing of nutrient intake and nutritional support over the day and in relation to sport rather than general daily targets.

The data implicated that the athlete has normal body composition parameters related to total body water (TBW= 52%), extracellular water (ECW=39%), intracellular water (ICW= 61%), along with body metabolic rate (BMR) which resulted 1943 (kcal/day). Based on the BIA, the athlete has 27% of fat mass and 31% of skeletal muscle, which can be seen in the attached figure of fat mass analysis. According to presented results of BIA, the ideal body weight for the athlete is 91.1 kg (min 88.8 kg, max 93.4 kg), which indicates that he is overweight.

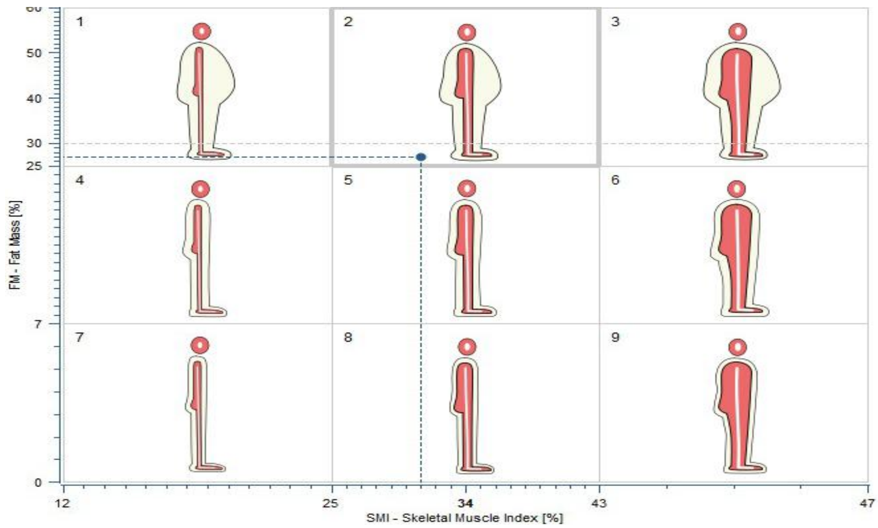

Figure 4. Skeletal Muscle Index- Fat Mass Analysis

CONCLUSION

Overall, we considered the athlete's previous food preferences and usual training cycle in order to adapt for an enormous intensity along with the purpose of maintaining an energy balance after the peak performance due to the 13 days of continuous physical activity. Moreover, we have used the guidelines per kg body mass for CHO and protein, although as mentioned, the athlete had opportunity to ingest additional calories considering the uniqueness of this event. In conclusion, the dietary plan was successfully developed for this challenge, in order to provide sufficient nutrients and to be adaptable considering the circumstances of this project. Finally, the dietary plan was successfully conducted throughout the marathon, enabling the athlete to use the most out of his physical potential.

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PREHRANA KAO ZNAČAJAN SEGMENT USPJEHA TRIATLONCA KOJI SUDJELUJE U PROJEKTU: HUMANOST KAO ZABORAVLJENA OLIMPIJSKA DISCIPLINA

Sažetak

Ovaj rad je dio projekta “Humanost kao zaboravljena olimpijska disciplina” koij predstavlja humanitarnu ekspediciju u kojoj će sportaš izdržati 13 dana triatlona sa startom na Poljoprivredno- prehrambenom fakultetu u Sarajevu, Bosna i Hercegovina, i krajnjim završetkom na vrhu planine Olimp u Grčkoj. Primarni cillj ovog projekta bio je prikupljanje finansijskih sredstva u svrhu liječenja oboljelih od raka. Svojim djelovanjem triatlonac se suočio s ekstremnim izazovima koji simbolično predstavljaju

bitku punu uspona i padova s kojima se oboljeli svakodnevno sukobljavaju. Triatlon je sport koji zahtijeva vještinu, trud, izdržljivost i motivaciju. Objedinjuje tri sportske discipline: trčanje, biciklizam i plivanje. Sportaš je bio angažiran u ovim disciplinama tokom svog poduhvata na planinu Olimp. Najvažniji aspekat ove humanitarne ekspedicije bila je snaga vlastite volje. Volja za uspijehom jednaka je volji za ozdravljenjem. U tim ekstremnim uslovima sportaš je imao podršku svog tima: koordinatora projekta, doktora medicine, nutricionistu, stručnjaka za planinarenje i fotografa. Zbog svakodnevne iscrpljujuće tjelesne aktivnosti, uloga nutricioniste je bila od izuzetne važnosti u ovom poduhvatu. Stoga je plan prehrane prilagođen uslovima boravka u kamperima i suočavanju sa neočekivanim izazovima.
Korištene metode su: Antropometrija (visina, težina, BMI); BIA; softver Program Prehrane 5.0; izrada plana prehrane za 13 dana uključujući prehrambene preferencije, energetske potrebe i parametre izdržljivosti. Očekivani ishodi odnose se na zadovoljavanje prehrambenih potreba sportaša s ciljem održavanja izdržljivosti i energije.

Ključne riječi: *humanost, triatlon, plan ishrane, izdržljivost, makronutrijenti*

THE ROLE OF PLANTS IN DIFFERENT ASPECTS OF HUMAN LIFE WITH FOCUS ON MENTAL HEALTH*

Jasna Avdić[1], Amra Kalabić[1], Berina Bečić[2], Alka Turalija[3]

Professional paper

Abstract

Urbanization leads to the exploitation and occupation of green areas, contributing to a high degree of their fragmentation. In this way, the habitats of plants and animals are endangered, which has a very negative effect on biodiversity within cities. These consequences of urbanization require innovative concepts of urban design that will increase the quality of life in urban areas.This paper presents ways of introducing plants into cities, and presents their importance in improving the quality of life in urban areas. Given that there is a higher percentage of mental health problems in cities than in rural areas, special emphasis is placed on the role of green areas and plants indoors in improving the mental state of people, and in preserving their mental health. The results of numerous researches that contribute to the understanding of the effect of plants on the quality of life in urban areas are given.

Key words: *urbanization, fragmentation of green areas, quality of life, mental state, mental health*

INTRODUCTION

The majority of residents of urban areas perceive the natural environment more positively than the urban environment and due to this fact, the construction or renovation of existing green areas is an indispensable item when forming plans for the arrangement of urban areas. Predictions of human population growth require a continuous process of urbanization at the global level (Alberti, 2017). Urbanization leads to the exploitation and occupation of green, contributing to a high degree of fragmentation, the loss of green in the areas of development and expansion of cities, the loss of connectivity of plant and animal habitats, and the reduction of biodiversity within cities. The aforementioned consequences of urbanization require innovative concepts of urban design that will increase the quality of life in urban areas, aimed at the development of more sustainable, resilient and healthy cities (Russo and Cirella, 2018). The results of numerous studies contribute to the understanding of the effect of plants on the quality of life in urban areas. Introducing plants through innovative concepts of urban design will increase the quality of life in urban areas. Given that there is a higher percentage of

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mental health problems in cities than in rural areas, special emphasis is placed on the role of green and indoor plants in improving the psychological state of a person, and in preserving his mental health.

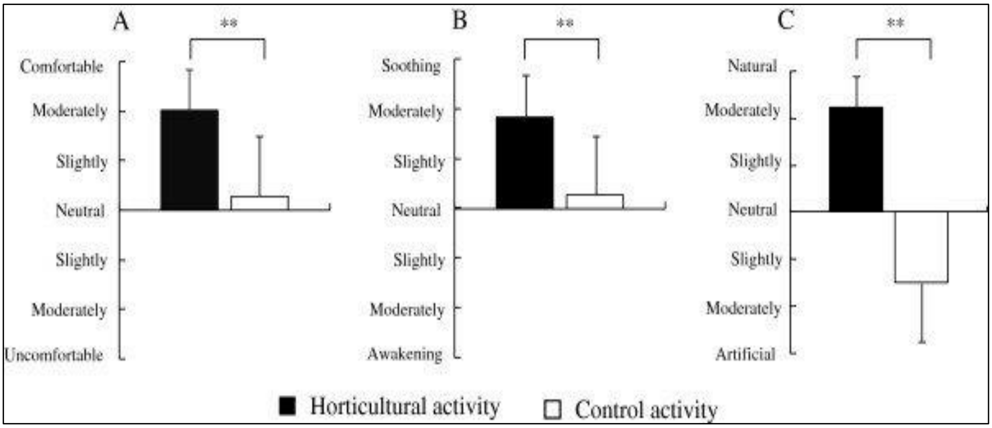
MATERIALS AND METHODS

The preparation of this work was preceded by an exhaustive analysis of collected data on the ways in which green and plants in the exterior and interior affect the lives of residents in an urban environment, and a special emphasis was placed on the psychological state, that is, on the positive effect of plants in preserving the mental health of the city population. The method of work applied in this research was implemented through the following stages: a) collection of information (study of literature), b) analysis of data from scientific and professional literature and c) unification of results.

RESULTS AND DISCUSSION

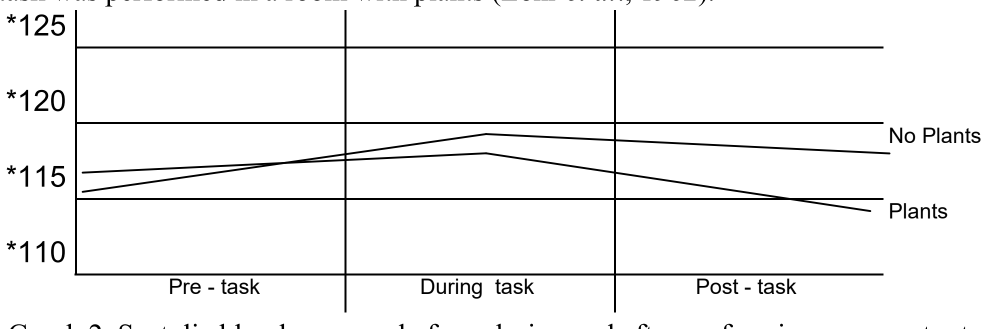
Ways of using plants for some specific problems, such as polluted air, have been researched in detail. However, the basis of intangible benefits, such as the effect of plants on mood, is still insufficiently researched, which is why there are certain unknowns in this sphere of plant action (Lohr, 2010). Through three main roles (aesthetic-decorative, sanitary-hygienic and cultural-educational), green and indoor plants contribute in many ways to better conditions for human life in the urban environment. The sanitary and hygienic role is also reflected in the fact that during the summer months plants increase the humidity of the air and lower their temperature by 2 to 3°C. When it was determined that plants have a positive effect on the mental health and psychological state of a person, research began to be conducted with the aim of explaining the specific mechanisms and ways of their action. Through research on the benefits of plants, there has been an increase in their use to solve environmental and health problems in preserving and improving the mental health of people in an urban environment.

Psychological relaxation (Lee *et al*., 2013) through the horticultural activity of transplanting real and artificial flowers yielded psychological responses measured using a semantic differential rating scale followed by measurements of heart rate and heart rate variability (HRV). The mental states of the participants during each activity were monitored by heart rate measurements and heart rate variability (HRV). Analysis of physiological responses using two-way analyses of variance (ANOVA) revealed that sympathetic nerve activity significantly decreased in the late period (11 to 15 minutes) of horticultural activity only in the group of participants who worked with real plants and not with artificial ones (Lee *et al*., 2013). Horticultural activity promotes pleasant, calm and natural feeling

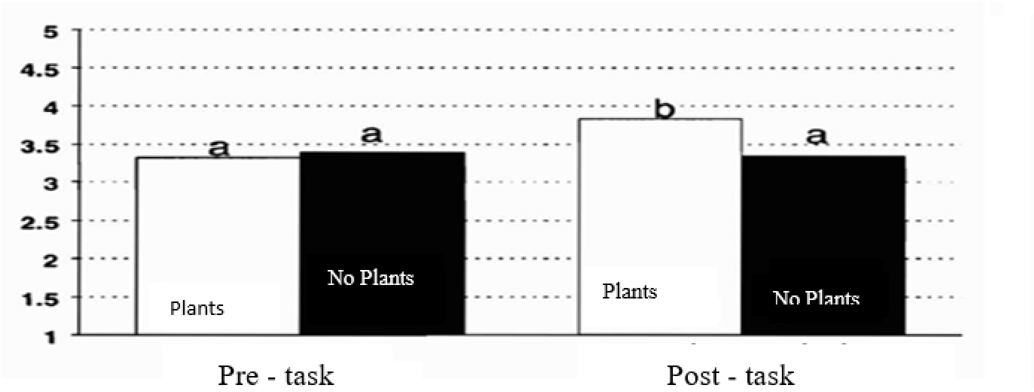


Graph 1. Comparison of changes in mental state after horticultural and control activities
A – Pleasant feeling; B – Feeling of calmness; C – Affection for plants (Lee *et al.,* 2013)

Research related to the role of plants in increasing productivity and reducing stress levels in the workplace shows that workers in work rooms without windows have less job satisfaction and rate the physical conditions of their work as "less pleasant and stimulating". Many people believe that bringing plants indoors improves worker productivity and satisfaction, but there is still relatively little research that specifically examines these impacts. Research conducted in a teaching computer lab (University of Washington) documents the benefits of bringing plants into a windowless workspace. Participants were divided into two groups, one of which performed the task in a room without plants, while the other group performed the task in the presence of plants. The blood pressure and emotions of the participants were monitored while they performed a simple computer task with the presence or absence of plants in the room monitored the speed of task performance, number of errors, and stress level. After the experiment, it was determined that productivity was higher and the stress level was lower when the task was performed in a room with plants (Lohr *et al*., 1962).

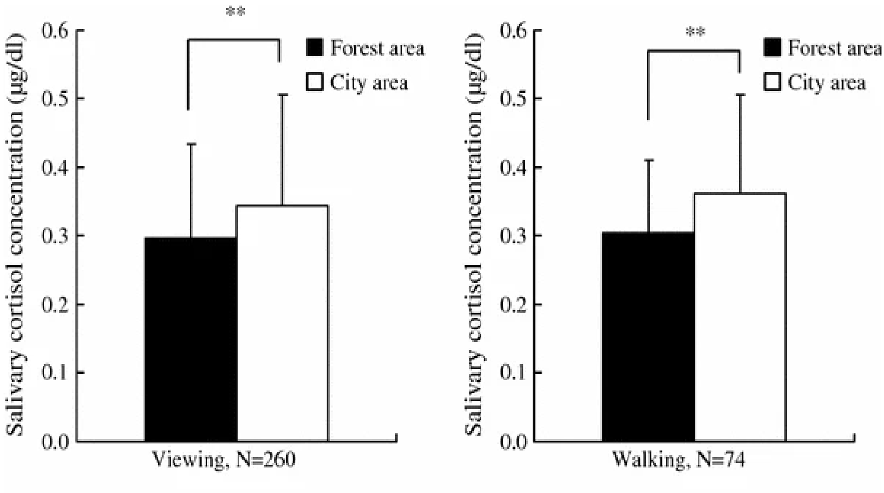


Graph 2. Systolic blood pressure before, during and after performing a computer task (Lohr *et al*., 1962)

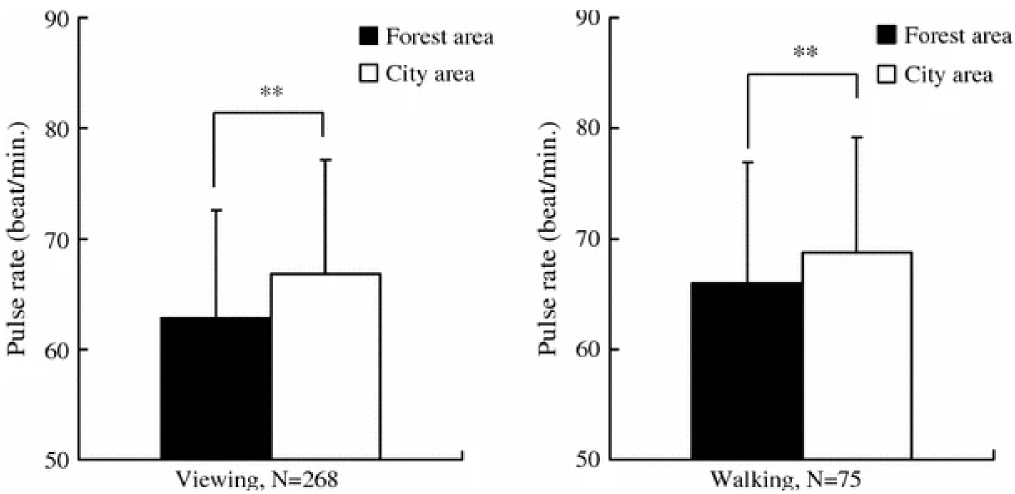


Graph 3. Errors and reaction time while performing a computer task with the presence or absence of plants (Lohr *et al*., 1962)
Graph 4. Respondents' answers to the statement "I feel concentrated" on a scale from 1 to 5, before and after performing the computer task with or without plants (Lohr *et al*., 1962)

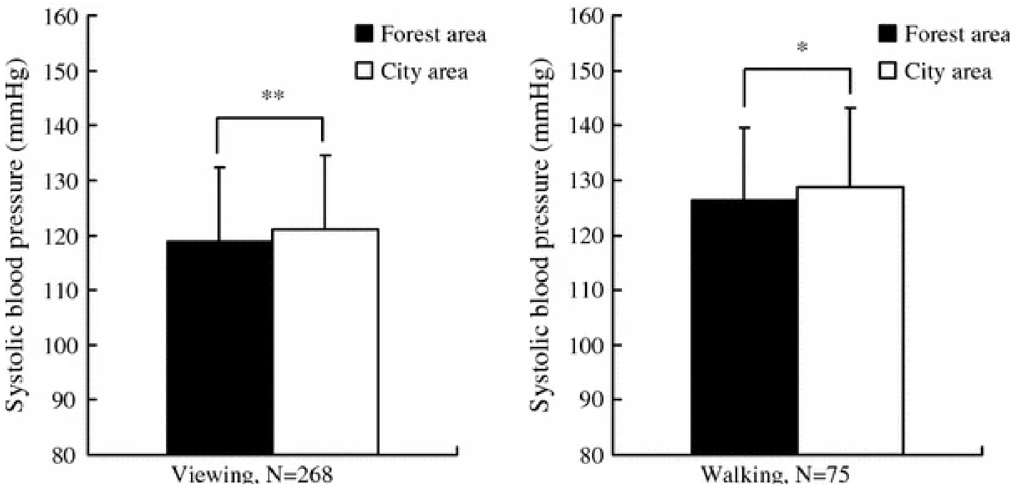
Research related to the physiological effects of Shinrin-yoku ("forest bathing") was carried out through field experiments conducted in 24 forests throughout Japan. During the experiments, the subjects went to the forest or the city, where they stayed for a certain period, stayed in rooms with the same conditions, and received the same meals. Cortisol, blood pressure, pulse and heart rate variability were used as indicators. On the first day, one group of respondents stayed in the forest, and the other respondents stayed in the city. The next day, each group was sent to a different area for cross-checking. The results showed that the forest environment promotes lower cortisol concentration, lower heart rate, lower blood pressure, higher parasympathetic nerve activity and lower sympathetic nerve activity than the urban environment. The relationship between the natural environment and relaxation (eg lowering blood pressure and pulse rate, inhibiting sympathetic nerve activity, increasing parasympathetic nerve activity and lowering cortisol levels). These results will contribute to the development of a research area dedicated to forest medicine, which can be used as a strategy for preventive medicine (Jin *et al*., 2009).



Graph
5. Change in cortisol concentration in saliva after staying in the forest (Jin *et al*., 2009)



Graph 6. Change in heart rate after staying in the forest (Jin *et al*., 2009)



Graph 7. Change in systolic blood pressure after staying in the forest (Jin *et al*., 2009)

Through numerous experimental studies, it has been proven that passive and active experiences of nature have a positive effect on the psychological and physiological well-being of humans and that they contribute to the preservation of mental health. However, any natural environment (a park or a promenade with trees) can contribute to a better mood and have a positive effect on mental health. Watching photos or videos with scenes from nature has a positive effect on the mood, encouraging positive emotions. The presence of plants in closed spaces is also extremely important because they make the space more comfortable and pleasant. It has been proven that people prefer to stay in rooms where there are plants. They purify the air and increase humidity, thus creating healthier conditions for living indoors. Although several experiments have been conducted to understand how plants affect the psychological state and mental health of humans, there is still not enough data to fully understand the mechanism of their action.

CONCLUSIONS

In order to mitigate the negative consequences of urbanization, it is necessary to focus on innovative solutions of urban design that will enable increasing the sustainability of cities and improving the quality of life in them. Until now, many experiments and research have been conducted documenting a wide range of positive effects of plants on humans. Various studies prove that human-plant interactions have a positive effect on human health. Research conducted with different target groups in different countries and in different circumstances (students, workers, patients, teenagers, elderly people) resulted in a positive response to the presence of plants, either in the form of active interaction or passive experience.

However, although a large amount of research has been conducted on the interaction between humans and plants, and on their effect on the mental health and psychological state of humans, there are still many unknowns when it comes to the mechanism of action of plants. The reason for this is the methodological limitations of the research, which make it difficult to investigate and fully clarify the effect of plants on mental health and emotional state in more detail. Although the specific way plants affect mental health and human emotions is still insufficiently researched, it can be concluded that contact with plants represents an intuitive action that has a positive effect on psychological stability by stimulating human senses in different ways.

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ULOGA BILJAKA U RAZLIČITIM ASPEKTIMA LJUDSKOG ŽIVOTA S AKCENTOM NA MENTALNO ZDRAVLJE

Sažetak

Urbanizacija dovodi do iskorištavanja i zauzimanja zelenih površina, doprinoseći visokom stepenu njihove fragmentacije. Na ovaj način dolazi do ugrožavanja staništa biljaka i životinja što izrazito negativno djeluje na biodiverzitet unutar gradova. Navedene posljedice urbanizacije zahtjevaju inovativne koncepte urbanog dizajna koji će povećati kvalitet života u gradskim sredinama.
U radu su navedeni načini unošenja biljaka u gradove, te je predstavljen njihov značaj u poboljšanju kvalitete života u urbanim sredinama. S obzirom da je u gradovima prisutan veći procenat problema mentalnog zdravlja nego u rualnim područjima, poseban akcenat je stavljen na ulogu zelenih površina i biljaka u zatvorenim prostorima u poboljšanju psihičkog stanja čovjeka, te u očuvanju njegovog mentalnog zdravlja. Navedeni su rezultati brojnih istraživanja koja doprinose shvatanju djelovanja biljaka na kvalitet života u gradskim sredinama.

Ključne riječi: *urbanizacija, fragmentacija zelenih površina, kvalitet života, psihičko stanje, mentalno zdravlje*

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IN MEMORIAM

Prof. dr. Sonja Bijeljac

Prof. dr Sonja Bijeljac rođena je 1947. godine u Beogradu. Upisala je Poljoprivredni fakultet u Sarajevu 1966. godine na kojem je diplomirala 1970. godine. Iste godine se, kao izuzetni student zapošljava kao asistent na Katedri za mljekarstvo Poljoprivrednog fakulteta u Sarajevu. Magistarski rad iz oblasti mljekarstva brani 1974. godine i stiče titulu Magistar poljoprivrednih nauka – proizvodnja i prerada mlijeka. Doktorirala je 1987. godine na Poljoprivrednom fakultetu u Sarajevu. U zvanje docenta na oblasti Proizvodnja i prerada mlijeka je birana 1984. godine. U zvanje vanredni profesor na istoj oblasti je birana 1990. godine. Početak opsade je zatiče u Sarajevu gdje ostaje skoro do kraja (1995) u opkoljenom Sarajevu rastrgana između profesionalnih obaveza i ljubavi prema svojoj djeci koja su u jeku ratnih dejstava izašli iz Sarajeva i otišli sa bakom u Sloveniju. Vanredni je profesor na predmetima Kontrola proizvoda i Poznavanje i prerada mlijeka bila od 1999. godine. Od 2006. godine je bila u trajnom zvanju redovni profesor sve do odlaska u penziju, 2012. godine. No, time ne završava aktivnu karijeru već biva birana u zvanje *Professor Emeritus* i nastavlja svoj nastavno-naučno-pedagoški rad sve do 2019. godine. Od 1999. do 2002. godine je rukovodila postdiplomskim studijem Tehnologija biljnih proizvoda, a od 2000. godine je rukovodila postdiplomskim studijem Tehnologija animalnih proizvoda. U dva mandata je bila Upravnik Instituta za tehnologiju poljoprivrednih i prehrambenih proizvoda (1990.-1995 i 2000.- 2004. godine). U tri mandata (od 2000. do 2006. godine) je bila prodekan za finansije Poljoprivredno-prehrambenog fakulteta u Sarajevu. Nastavno-pedagošku aktivnost je obilježila velikim brojem mentorstava diplomskim i magistarskim radovima, doktorskim disertacijama, završnim radovima I i II ciklusa, specijalističkim radovima, te kao član komisija. Naučnu aktivnost je realizirala kroz autorstvo ili koautorstvo velikog broja naučnih i stručnih radova, knjiga, udžbenika,

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Bila je: Član Komisije za nostrifikaciju Univerziteta u Sarajevu; Član Izdavačkog savjeta za univerzitetska izdanja Univerziteta u Sarajevu; Član Odbora za etička pitanja Univerziteta u Sarajevu; Predsjednik Tehničkog komiteta za hranu – BAS/TC 43, Institut za standarde, mjeriteljstvo i intelektualno vlasništvo BiH, od 2005. godine; Član Tehničkog komiteta za poljoprivredu – BAS/TC 45, Institut za standarde, mjeriteljstvo i intelektualno vlasništvo BiH, od 2008. godine; Predsjednik Savjeta Instituta za standarde, od 2009. godina; Konsultant TAM-BAS-a od 2007. do 2009. godine; Član Naučnog odbora naučno-stručnog skupa sa međunarodnim sudjelovanjem - QUALITY 2005-2011. godine; Član interresorne grupe za izradu pravilnika o kvalitetu hrane pri Agenciji za sigurnost hrane 2008-2010. godine; Predsjednik komisije za ocjenu kvaliteta prehrambenih proizvoda na Međunarodnom sajmu poljoprivrede i prehrambene industrije - sajam šljive, Gradačac 2002-2011. godine; Predsjednik komisije za ocjenu kvaliteta prehrambenih proizvoda na Zeničkom privrednom sajmu – ZEPS, Zenica 2003-2011. godine; Predsjednik komisije za ocjenu kvaliteta autohtonih sireva BiH u organizaciji Udruge proizvođača i prerađivača autohtonih ovčjih sireva i mesa – Pramenka, Mostar 2002-2011. godine; Predsjednik komisije za ocjenu kvaliteta Vlašićkog sira. Ocjenjivačka izložba Vlašić 2006-2011. godine; Predsjednik komisije za ocjenu kvalitete autohtonih sireva na manifestaciji Dani šljive – Rama-Prozor 2006-2010. godine; Predsjednik komisije za ocjenu kvaliteta prehrambenih proizvoda na privrednoj manifestaciji Dani jabuke, Goražde 2005-2011. godine; Član Nadzornog odbora Udruženja agronoma FBiH.
Dobitnik je šestoaprilske nagrade grada Sarajeva i grupne nagrade za višegodišnji rad i rezultate iz oblasti laktologije.
Izuzetno cijenjena i poštovana u kolektivu, Prof. Sonja Bijeljac bila je uzorna osoba, puna kolegijalnosti, staložena kada je trebalo nekoga saslušati, dati savjet ili podršku ali i odlučna uvijek kada je trebalo donijeti neke odluke ili obaviti važne zadatke. Poznata je bila po svojoj energiji i ljubavi sa kojom je obavljala sve svoje dužnosti i poslove kao i po pozitivnim vibracijama i atmosferi kojima je zračila gdje god je bila. Bila je sjajan pedagog, nastavnik, naučnik, saradnik, šef, lider, voditelj programa, vjeran i uzoran član rukovodstva fakulteta. Bila je temeljita u svemu što radi.
Prof. Bijeljac je bila aktivna i u radu sa proizvođačima sira. Bila je Predsjednik svih komisija za senzornu ocjenu, gotovo do prije koju godinu i nije se libila niti da pohvali ali niti da kritikuje i održi lekciju proizvođačima sira kako trebaju raditi na popravci kvaliteta sira i higijeni.
Bila je jednostavno ČOVJEK široke i tople duše. Kao malo koga, krasili su je elokvencija, pismenost i visok nivo opšteg obrazovanja da teško da se iko mogao porediti sa njom.
Bila je izuzetan student da se godinama i generacijama pričalo o njoj. Čak je i pokojna prof. Dozet koja je bila poznata po pravednosti ali i strogoći i pomalo, pedagoški, škrta

na pohvalama, izjavila kada bi se pojavio neko ko bi se izuzetno isticao, bila je poznata njena rečenica „......još od Sonje Bijeljac.... nije bilo takvog studenta“.
A sada bi htio nešto reći što je najvažnije i što je možda trebalo ići na početak. O nečemu što mnogi od nas ne znamo ili manje znamo. Imala je prof. Bijeljac uspješan životni put ne samo u profesionalnom životu već i u privatnom.
Bila je dobra i brižna majka i baka.
Kako je nastojala da svoje vrijednosti prenese na studente koji su je voljeli i poštovali tako ih je uspjela prenijeti na svoja dva dragulja i najveće dostignuće, sinove Milana i Mlađena, vrijednosti čestitosti, marljivosti i poštenja i ugraditi dobar i iskren odnos među njima, koji je bitan naročito sada kad su nažalost ostali sami, bez najvećeg prijatelja.
Bila je staložena osoba i oslonac i pomoć ljudima oko sebe i često je pomagala, pa makar savjetom. Bila je brižna i predana majka, koja je pored uspješne akademsko naučne karijere uvijek posvetila svoje vrijeme dijeci i porodici. U toku pa i poslije rata bila je rascijepljena između Sarajeva i Ljubljane....Sarajeva gdje je imala posao i život, te Ljubljane gdje su joj djeca živjela. I kad je otišla u penziju, odlučila se je da ipak ostane u svom gradu.
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Imao sam posebnu čast i privilegiju da dobro poznajem prof. Bijeljac i budem njen blizak saradnik cijelim tokom moje radne karijere. Lično, bila mi je uzor, još od studentskih dana kada nam je držala praktičnu i teoretsku nastavu koja je bila savršeno organizovana i nije bilo šanse da je propustimo, preko predavanja na postdiplomskom studiju, pa do mog zaposlenja na Katedri za mljekarstvo na Poljoprivrednom fakultetu. Bila je iskren prijatelj i podrška kad god je trebalo, blag i razuman šef. Bila je član svih mojih komisija, na diplomskom, magistarskom i na doktoratu gdje su njene sugestije i pomoć bili dragocjeni. Izuzetno sam ponosan na preko 30 godina zajedničkog rada. Hvala vam profesorice za sve što ste me naučili!
Neka je vječna hvala i slava profesorici dr. Sonji Bijeljac!

Prof. dr. Zlatan Sarić

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