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AGRONOMSKI FAKULTET U ČAČKU

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FACULTY OF AGRONOMY
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1st INTERNATIONAL SYMPOSIUM ON BIOTECHNOLOGY

17–18 March 2023

Faculty of Agronomy in Čačak, University of Kragujevac, Serbia

- PROCEEDINGS -

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- PROCEEDINGS -

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PREFACE

"The scientific man does not aim at an immediate result. He does not expect that his advanced ideas will be readily taken up. His work is like that of the planter - for the future. His duty is to lay the foundation for those who are to come, and point the way."

Nikola Tesla

Agriculture is a primary and strategic activity that ensures food security and food market stability, and protects living standards for people. We have witnessed that, in crisis situations in the world, agriculture has responded to its task as the main support in supplying the market with food products. The production of sufficient quantities of safe food enables the development of the working-age population that actively participates in the economic development of society. The specific conditions in which agriculture develops require economic support measures and subsidies from the state to preserve domestic agriculture. Planned investments in agriculture through the introduction of modern technologies and efficient organization in both production and trade reduce economic and market risks, thus enabling stable business conditions. The development of agriculture must be based on a multifunctional connection with other activities (food industry, trade, tourism, etc.)

Agricultural science and agriculture as a profession monitor and study changes occurring in this area, point out problems in agricultural practice, and find solutions. The Faculty of Agronomy in Čačak, in addition to educating students, traditionally organizes the Symposium on Biotechnology every year. This year marks the 28th anniversary of the Symposium. The main goal of the Symposium is to acquaint the wider scientific and professional public with the results of the latest scientific research, and bring together domestic and foreign scientists in the fields of primary agricultural production, food processing, and environmental protection.

At the 1st International Symposium on Biotechnology, a total of 71 papers were presented in the fields of Field, Vegetable and Forage Crops, Pomology and Viticulture, Livestock Production, Plant Protection, Food Safety and the Environment, Food Technology and Applied Chemistry.

We owe great gratitude to the **Ministry of Science, Technological Development and Innovation of the Republic of Serbia** and the **City of Čačak** for their financial support and patronage to this Symposium, which they provide every year. We thank companies, entrepreneurs, stakeholders and all

long-time friends of the Faculty of Agriculture for their material and organizational support.

Doing agriculture, in addition to economy and business, is also a noble social activity, considering that it satisfies people's basic daily needs of food. Agricultural producers deserve reputation and respect in society and should be enabled to make a decent living from their work, and society should recognize this.

In Čačak, March 2023

Programme and Organizing Committee
1st INTERNATIONAL SYMPOSIUM
ON BIOTECHNOLOGY
(28th SYMPOSIUM ON BIOTECHNOLOGY
with international participation)

Faculty of Agronomy in Čačak
University of Kragujevac

is organizing

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17–18 March 2023, Čačak, Republic of Serbia

in cooperation with

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MAPK$_2$ AND NRAMP$_6$ EXPRESSION ANALYSIS UNDER CD STRESS IN DOMESTIC KALE VARIETIES FROM BiH

Jasmin Šutković[1], Annissa Van Wieren[2], Ahmet Yildirim[3]

Abstract: *Brassica oleracea var. acephala* (kale) is a worldwide known vegetable plant, known for its heavy metal accumulation abilities. This study analyzed the phytoremediation potential of domestic BiH kale varieties, grown in a controlled environment and with different concentrations of Cadmium (Cd). Cd is a known pollutant found in small concentrations in soil under normal environmental conditions. Real-Time PCR was used to analyze the gene expression activity of Cd heavy metal correlated genes (MAPK$_2$ and NRAMP$_6$). The root analysis assay confirmed shorter root length after Cd treatment, confirming severe Cd impact on plant cell growth, implying shorter growth in roots in all treated kales. Gene expression results showed that Cd triggers the expression of MAPK$_2$ and NRAMP6 suggesting their significant involvement in Cd metabolizing processes. Based on the combined results, kale from the Stolac region is found to be the most resistant variety, while the only hybrid kale, accumulates the most Cd suggesting its phytoremediation potential.

Keywords: *Brassica oleracea*, heavy metals, kale, cadmium, phytoremediation

Introduction

Brassicaceae, also known as Cruciferae or mustard family, is one the largest dicot families that belong to angiospermic plants and covers 10-19 tribes with approximately 3,709 species and a total of 338–360 genera (Anjum et al., 2012). Brassicaceae plants are particularly important in agriculture, horticulture, and the economy as they have many vitamins such as vitamins A, C, E, K, and minerals.

In addition, Brassica plants represent the main sources of condiments, vegetable oil, and vegetables (El-Esawi et al., 2017). *Brassica oleracea* is a popular

[1]International University of Sarajevo, Faculty of Engineeiring and Natural Sciences, Hrasnička cesta 15, Ilidža , Bosnia and Herzegovina (jsutkovic@ius.edu.ba)

[2]International University of Sarajevo, Faculty of Engineeiring and Natural Sciences, Hrasnička cesta 15, Ilidža , Bosnia and Herzegovina (210301011@student.ius.edu.ba)

[3]International University of Sarajevo, Faculty of Engineeiring and Natural Sciences, Hrasnička cesta 15, Ilidža , Bosnia and Herzegovina (ayildirim@ius.edu.ba)

kale that originated from South Asia and is considered as one of the healthiest foods with antioxidant, and anti-cancerogenic properties gastrointestinal (GI) tract and circulation system (Ortega-Hernández et al., 2021). It is confirmed that *B. Oleracea* is a phytoextraction plant as it can accumulate heavy metals in their tissues, without any visible symptoms (Šabanović et al., 2020).

Among 90 natural elements, several of them are accounted as heavy metals, such as cadmium (Cd), Fluorine (F), and lead (Pb), being harmful and nonessential (Hejna et al., 2018). In plants, toxic heavy metals and even excess essential heavy metals may result in chlorosis, malnutrition, and diminished development. However, some of these plants and other organisms accumulate heavy metals in excess. The ability of plants to accumulate heavy metals is known as phytoremediation. Phytoremediation is not always effective but at least it is less harmful than using chemical treatments such as membrane filtration and soil washing, and physical treatments (Murtić et al., 2021).

A study conducted in 2016, confirmed and predicted novel genes correlated to Cd stress, and among many, Mitogen-activated protein kinase 2 ($MAPK_2$) and Natural resistance-associated macrophage protein 6 ($NRAMP_6$) were correlated to known Heavy metal accumulator 2 (HMA_2) gene in *B. oleracea*. (Šutković et al., 2016). Another name for $MAPK_2$ is extracellular signal-regulated kinase 2 (ERK_2) and it is involved in many cellular pathways such as cell proliferation. It is usually induced in response to various stimuli, such as hormones and growth factors (GF) (Li et al., 2022).

$NRAMP_6$ is part of metal transporter proteins and is present in many organisms such as bacteria, fungi, and plants. Like every natural resistance-associated macrophage protein (NRAMPs), they are capable of transporting bivalent metal ions such as Fe^{2+} and Mn^{2+} into the cytoplasm. It is determined that $NRAMP_6$ transports excess Cd ions intracellularly in *A. thaliana* (Ullah et al., 2018) and it enables plants to develop normally with inadequate nutrients such as Mn^{2+}.

Through root assay and RT-PCR gene expression analysis, we aimed to analyze the phytoremediation potential of 2 domestic and 1 hybrid kale, in response to different Cd concentrations.

Materials and methods

Plant growth and treatment

In the study, two domestic kale varieties (marked as kale S1 and kale M) and one hybrid kale (kale 23) was used, as seen in table 1.

Table 1. Kale varieties used in this study

Regions (Country, city, village)	Geographical coordinates	Code/lable
BiH, Mostar	43°25 North, 17°88 East	KM
BiH, Stolac	44°82 North, 18°57 East	KS1
Brassica oleracea L. - Bonanza F1	Italy	K23

Brassica oleracea var. acephala seedlings were grown using Tap-of-paper method. Seeds were treated with different concentrations of Cd (control – distillated water, 100 µM, 250µM, 500µM and 1000µM of $CdCl_2$) and incubated for 5 days at 27 ºC in growth chamber where they were exposed to light for 16 hours a day, according the protocols explained by Šutković et al., 2022.

Primer design

Primer design for the annotated genes in *Brassica oleracea*, as shown in table 2, were done with an incorporated NCBI server tool, called PRIMER-BLAST (Ye et al., 2012).

Table 2. Oligonucleotides used in the present research

Genes	Sequences (5'->3')
F- MAPK2	GGGCTGCCAAAGGACTTACT
R- MAPK2	GTCTTGTCACCGGTAGGACC
F- NRAMP6	GCGATATCTCTCCTCGGTGC
R- NRAMP6	AAGCTTCCTTGATGCCGGTT
F-UBQ2	ATATTCGTGAAGACGCTG
R-UBQ2	CTCAACTGGTTGCTGTG

RNA isolation and DNAase treatment

RNA isolation and DNase treatment were performed with Monarch® Total RNA Miniprep Kit protocol (NEB #T2010). According to the kit manufacturer, two main steps were performed. The first step was that the plant tissues were lysed, and the second step was that RNA was purified (Dotti and Bonin 2011).

qPCR amplification

qPCR amplification was performed on the Step One Plus system by Applied Biosystems®.The qPCR reaction was prepared using the Luna Universal One Step RT-qPCR kit from NEB, according to manufacturer's instructions. Gene expression and statistical analysis were peformed according the protocol published by Šutković et.al, 2022.

Results and discussion

The average root length of kale 1 can be observed in Figure 1. The root length decreased with the increase of $CdCl_2$ concentrations. In the control group, root length ranged from 2.54cm to 3.73cm, while in 1000µM $CdCl_2$ it ranged from 0.54cm to 0,86cm.Tukeys multiple comparisons test showed that there was not a significant difference in the mean root length between the all concentrations used in the study, $p < 0.05$.

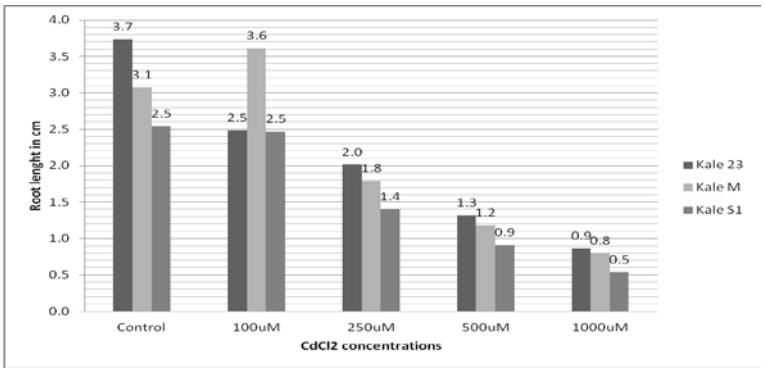

Figure 1. The average root length in *Brassica oleracea* varieties under different $CdCl_2$ concentrations.

The results showed that there was an overall relative decrease in root length with increasing Cd concentration, with is no significant difference in lights among varieties. These results are in line with the study conducted by Waheed et al., 2022, in which the heavy metals are accumulated in the leaves of *Eruca sativa* resulting in shorter roots and shoots. These observations are in agreement with the results of Ahmad et al. (2015), where Cd affected most of the growth parameters in *Brassica juncea* and *B.napus*, decreased shoot and root length. Similar results were reported in the studies conducted by Dutta et al. (2018), reducing root growth and shoot lengths in *B. juncea* under different heavy metals.

Real-time PCR was used to determine the relative expression of the

$MAPK_2$ and $NRAMP_6$ genes, with 2-5 replicates. Data are presented graphically where Y-axis in graphs represents $2^{-\Delta\Delta Ct}$ which basically analyzes the relative changes in gene expression.

The effect of Cd on $MAPK_2$ and $NRAMP_6$ gene expression is shown in Figure 2 to Figure 4. Different expression patterns were observed at different

concentrations. There is a small nonlinear increase in gene expression of $RAMP_6$ levels by increasing Cd concentrations in all kales. $MAPK_2$ increases in the domestic varieties but decreases in the hybrid variety. Calliatte et al., 2009 reported that $NRAMP_6$ was found to interfere with Cd in yeast cells. However, what is interesting is that there was an overall decrease expression of $MAPK_2$ in Kale 23. It is possible that these plants were dying due to higher concentrations of toxic heavy metals or that mutations may occurred in $MAPK_2$ primers during PCR reaction (Qiu et al., 2001) as there was study in *A. thalia*na and yeast cells found that L157P mutation affects how Cd is transported (Lu 2020).

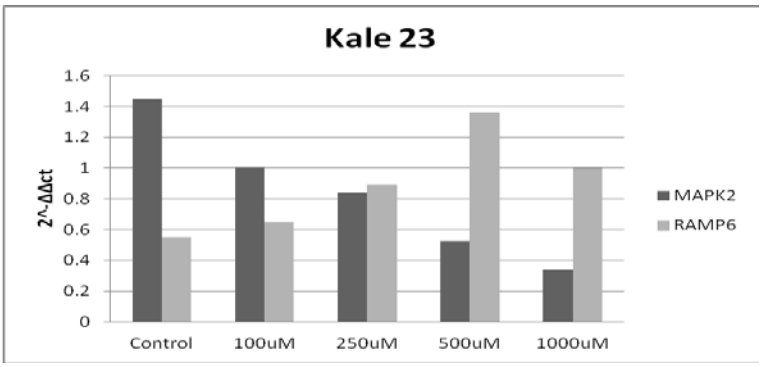

Figure 2. The effect of Cd on relative expression levels of MAPK2 and NRAMP6 genes in Kale 23.

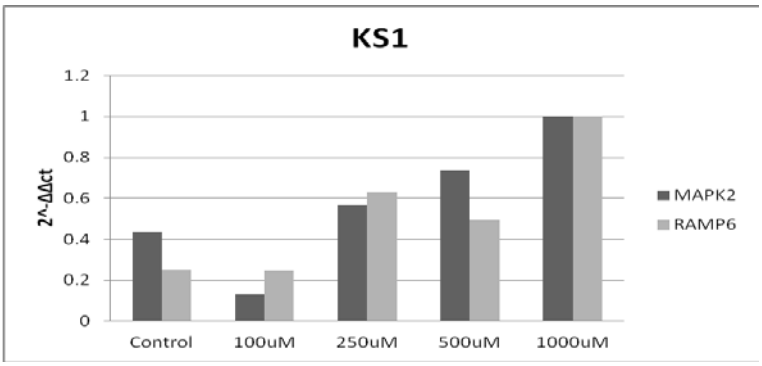

Figure 3. The effect of Cd on relative expression levels of MAPK2 and NRAMP6 genes in Kale S1.

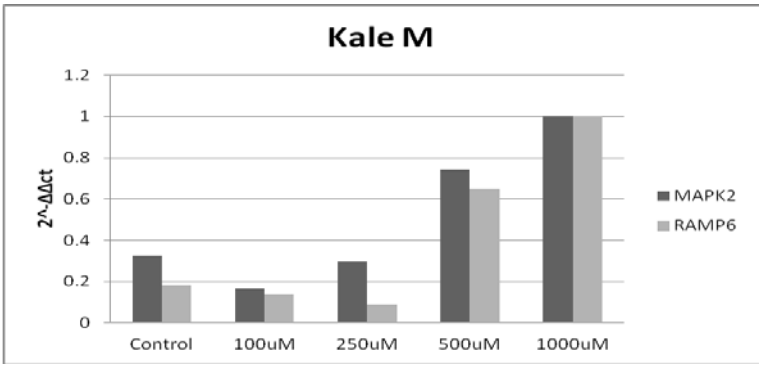

Figure 4. The effect of Cd on relative expression levels of MAPK2 and NRAMP6 genes in Kale M.

However, in kale S1 and M, the expression of $MAPK_2$ was increasing with higher concentrations of Cd. Similar results were observed in Kale 23, wehere the expression of $NRAMP_6$ increased, increasing linearly up to 500 µm $CdCl_2$ band declined at the 1000µM $CdCl_2$ concentration. The results of $NRAMP_6$ expression in Kale M and S1 are nonlinear e.g., there is least expression of $NRAMP_6$ at 1000 µM $CdCl_2$, indicating a potential cell entering into apoptosis (Siddiqui et al., 2022). This study represents an initial attempt to correlate $NRAMP_6$ and $MAPK_2$ gene expressions with cadmium accumulation in *B. oleracea species*. However, further analysis should be undertaken to additionally

Confirm the correlation of cadmium $NRAMP_6$ and $MAPK_2$ gene expression levels.

Conclusion

Based on the obtained root length and gene expression data, it can be concluded that Kale S1 is the most resistant kale out of all three kales and all experiments prove that it accumulates the least amount of Cd. The results from RT-qPCR showed that there was limited gene expression in Kale S1, if compared to other kales. Based on the obtained data, we can conclude that Kale 23 ccumulated most amount of Cd and therefore indicating that Kale 23 would be the best candidate for phytoremediation, while Kale S1 would be the healthiest kale for consumption.

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THE INFLUENCE OF THE TIME OF BASIC TILLAGE AND FERTILIZATION ON SOYBEAN YIELD

Vojin Đukić[1], Jegor Miladinović[1], Gordana Dozet[2], Marija Bajagić[3], Gorica Cvijanović[4], Zlatica Mamlić[1], Vojin Cvijanović[5]

Abstract: The time of basic tillage and fertilization are very important agrotechnical measures that have a high impact on soybean yield. Winter basic tillage of the soil reduces the harvest yield by 8.53%, and spring tillage by 21.18%, the application of NPK fertilizers increased the yield by 13.96%, the foliar application of water extract from plant material by 9.57% and the application of AN by 8.44%. Autumn basic tillage is most conducive to achieving high and stable soybean yields, and fertilization has a positive effect on soybean yields.

Keywords: basic tillage, soybean fertilization, NPK fertilizer, water extract of plant material, AN.

Introduction

To achieve high and stable soybean yields, it is necessary to apply all agrotechnical measures correctly and in a timely manner (Đukić i sar., 2018), but we must bear in mind that the most important agronomic and chemical properties of each variety are strongly influenced by external environmental factors and are subject to changes in depending on climate and soil conditions (Miladinović i sar., 2013). Basic tillage and pre-sowing preparation of the soil are very important agrotechnical measures that participate with about 20% in the total amount of yield achieved (Khurshid i sar., 2006) and affect the sustainable use of the soil through the influence on its properties (Lal, 2013). Spring basic tillage affects yield reduction and deterioration of soil quality, while autumn basic tillage reduces soil compaction due to more favorable soil moisture for tillage and more favorable temperature conditions (Al Kaisi i Hanna, 2010). In an unfavorable year for soybean production, when low

[1]Field and Vegetable Crops Institute, Maksima Gorkog 30, 21000 Novi Sad, Serbia (vojin.djukic@ifvcns.ns.ac.rs)

[2]Megatrend University, Faculty of Biofarming, M. Tita 39, Bačka Topola, Serbia

[3]University Bijeljina, Faculty of Agruculture, Pavlovića put bb, Bijeljina, Bosna and Hercegovina

[4] University of Kragujevac, Institute for information Technologies, Jovana Cvijića bb, Kragujevac, Serbia

[5]Institute for Science Application in Agriculture, Bulevar Despota Stefana 68b, Beograd, Serbia

average yields are achieved, spring basic tillage has a very large impact on the reduction of soybean yields, while in favorable years, without a marked lack of precipitation, spring basic tillage has a small impact on reducing soybean yields (Adee, 2018) In the case of spring tillage compared to autumn basic tillage, the yield of soybeans in a favorable year for soybean production was reduced by 7.41%, while in a dry, unfavorable year, the yield was reduced by 36.41% (Dozet i sar., 2018). Autumn basic processing is a prerequisite for achieving high yields of soybeans, and delays in the implementation of this agrotechnical measure statistically significantly reduce the yield, especially in unfavorable years with a pronounced dry period (Bajagić i sar., 2022).

Aqueous extracts of plant material are increasingly used in plant production, floriculture, vegetable growing, but also in agriculture, both in organic and conventional production (Đukić i sar., 2021). Foliar fertilizers contain elements that are easily absorbed by plants, and their effectiveness depends on the amount of nutrients in the soil, the plants' need for certain elements, the condition of the crops and the time of application (Miladinov i sar., 2018; Cvijanović i sar., 2022).

Fertilizing soybeans with different fertilizers continuously represents a research challenge (Miladinov et al., 2018). For the correct fertilization of soybean crops, we must know the characteristics and agrochemical properties of the soil, the plants' nutrient requirements, climatic conditions, crop rotation, application of manure and mineral fertilizers, plowing of the harvest residues of the pre-crops, the intensity of production on the plot in previous years, the yield of the pre-crops and the expected yield of soybeans (Mamlić i sar., 2021). Aqueous extracts of plant material, in addition to macro and microelements, also possess physiologically active substances that stimulate the growth and development of plants, often have fungicidal and insecticidal effects, are easily prepared on the farm, do not require large investments and are suitable for organic production since there is no negative effect on the environment (Mamlić i sar., 2022). Nettle is suitable for making extracts because it has fungicidal and insecticidal effects, and when fermented it becomes a significant source of nutrients for plant nutrition through feeding (Dozet i sar., 2019), it also contains growth stimulators (Di Virgilio 2013), and the banana fruit is rich in potassium, phosphorus, calcium, manganese, magnesium, selenium, contains vitamins C and B and vitamin A.

The aim of these researches is to assess the influence of the time of basic tillage and the influence of fertilization on soybean yield.

Materials and methods

A two-year experiment on the influence of the time of basic tillage and fertilization on soybean yield was conducted in 2020 and 2021 on a private plot in the vicinity of Bač, Bačka region, Vojvodina. In the experiment, there were three variants of the basic processing time: autumn in October, winter in January and spring in March. The four subvariants with fertilization were: the control variant without fertilization, the application of NPK fertilizer formulation 8:15:15 in the amount of 300 kgha^{-1}, the application of AN in the amount of 150 kgha^{-1} and the variant with the application of a diluted aqueous extract of nettle, comfrey and banana fruit, in the amount of 450 litaraha^{-1}. NPK fertilizer was applied immediately before the basic tillage, and nitrogen fertilizer AN with pre-sowing soil preparation. The aqueous extract was prepared by chopping the above-ground part of nettle (250 g), the above-ground part of comfrey (250 g) and banana fruits (500 g) and 10 l of water was added to the plant material. The container for the fermentation of plant material was placed in the shade and mixing of the plant material in the container was done every day. After 20 days, at the end of fermentation, the aqueous extract was filtered through cheesecloth, and during foliar application, the aqueous extract was diluted in a ratio of 1:15. Foliar application of the aqueous extract was carried out in the vegetative phase, before the flowering of soybean plants. The experiment was set up in 4 repetitions and the mid-late variety Rubin from the II ripening group was used. uring the growing season, the standard technology for growing soybeans was applied, and in the stage of technological maturity, harvesting, measurement of samples and grain moisture, and yield calculation with 14% moisture were performed. The results were processed statistically in the "Statistica 10" program, and the significance of the results was tested using the LSD test. The results are tabulated.

Results and discussion

The influence of the time of basic tillage and fertilization on soybean yield is shown in table 1.

Looking at the years of research, it can be seen that the average yield in 2020 (2853 kgha^{-1}) is statistically significantly higher than the yield achieved in 2021 (2485 kgha^{-1}).

Observing the average yields according to the time of basic tillage, it can be noted that the highest yield was achieved in the autumn basic tillage (2955 kgha^{-1}), which is statistically very significantly higher than the winter basic

tillage (2725 kgha^{-1}) and the spring basic tillage (2329 kgha^{-1}). Statistically very significant differences in yield were also between winter and spring basic tillage.

According to the fertilization variants, it can be observed that the highest soybean yield was achieved with the application of NPK fertilizer (2809 kgha^{-1}), which is statistically very significantly higher than the other fertilization variants (control 2495 kgha^{-1}, foliar application of water extract from plant material 2701 kgha^{-1} and application of nitrogen fertilizer AN 2673 kgha^{-1}). A statistically very significantly higher value for soybean yield was achieved in the variants with the application of aqueous extract from plant material and in the application of nitrogen fertilizer AN compared to the control variant of the experiment.

Table 1. The influence of the time of basic tillage and fertilization on soybean yield (kgha^{-1})

Year (A)	Time of basic tillage (B)	Fertilization (C)				Average AxB	Average A
		Control	NPK	AN	Foliar		
2020	Autumn	2867	3280	3204	3196	3137	2853
	Winter	2643	3014	2905	2907	2867	
	Spring	2408	2646	2591	2574	2555	
	Average AxC	2640	2980	2900	2892		
2021	Autumn	2605	2935	2767	2783	2772	2471
	Winter	2352	2711	2448	2641	2538	
	Spring	1914	2267	2122	2106	2102	
	Average AxC	2291	2638	2446	2510		
Average BxC	Autumn	2736	3107	2985	2989	Average B	2955
	Winter	2498	2862	2676	2774		2703
	Spring	2161	2457	2356	2340		2329
	Average C	2465	2809	2673	2701	-	-
Average 2020-2021							2662

LSD	A	B	C	AxB	AxC	BxC	AxBxC
1%	110,5	57,8	68,8	89,3	106,5	122,0	175,0
5%	48,2	33,0	41,6	50,7	63,8	73,8	105,9

Observing the same year and different time of basic tillage, it can be seen that in 2020 the highest yield was achieved on the trial variants with autumn basic tillage (3137 kgh^{-1}), which is statistically very significantly higher value compared to winter basic tillage (2867 kgha^{-1}) and spring basic tillage (2555

kgha^{-1}). Statistically very significant differences also existed between the values recorded on the variants with winter basic tillage and spring basic tillage. In 2021, the highest yield was achieved on the trial variants with autumn basic tillage (2772 kgh^{-1}), which is statistically very significantly higher value compared to winter basic tillage (2583 kgha^{-1}) and spring basic tillage (2102 kgha^{-1}). Statistically very significant differences also existed between the values recorded on the variants with winter basic tillage and spring basic tillage.

Looking at the same year and different fertilizing variants, it is noted that the highest yield of soybeans in 2020 was achieved on the variant with the application of NPK fertilizer (2980 kgha^{-1}), which is in addition to the yields on the variants with the application of nitrogen fertilizer AN (2900 kgha^{-1}) and foliar application of water extract (2892 kgha^{-1}) statistically very significantly higher value compared to the control variant (2640 kgha^{-1}). With the application of NPK fertilizer, the yield of soybeans was statistically significantly higher compared to the application of nitrogen fertilizer AN and the application of water extract from plant material. In 2021, the yields of soybeans on variants with the application of NPK fertilizer (2638 kgha^{-1}) and water extract from plant material (2510 kgha^{-1}) were statistically very significantly higher compared to the control variant of the experiment (2350 kgha^{-1}), while with the application of nitrogen fertilizer AN yields are statistically significantly higher (2446 kgha^{-1}). A statistically very significantly higher yield was also achieved on the variant with the application of NPK fertilizer compared to the variants with the application of aqueous extract from plant material and nitrogen fertilizer AN, and statistically significantly higher yield was also obtained with the application of aqueous extract from plant material compared to the application of nitrogen fertilizers AN.

Observing the same time of the basic tillage of the soil and different variants of fertilization, it is observed that in the autumn basic tillage the highest yield was recorded with the application of NPK fertilizer (3107 kgha^{-1}), which is statistically very significantly higher yield compared to the control variant of the experiment (2736 kgha^{-1}) and the application of nitrogen fertilizer AN (2985 kgha^{-1}) and a statistically significantly higher yield compared to the application of aqueous extract from plant material (2989 kgha^{-1}). Compared to the control variant of the experiment, a statistically very significantly higher yield was also achieved on the variants with the application of aqueous extract from plant material and nitrogen fertilizer AN. In the winter basic tillage, the lowest yield of soybeans was achieved in the control variant of the trial (2587 kgha^{-1}), which is a statistically very significantly lower value compared to the application of

NPK fertilizers (2862 kgha^{-1}), water extract from plant material (2774 kgha^{-1}) and nitrogen fertilizer AN (2676 kgha^{-1}). A statistically very significantly higher yield was achieved with the application of NPK fertilizer compared to the application of AN nitrogen fertilizer and a statistically significantly higher yield compared to the application of aqueous extract from plant material. In the spring basic tillage with the application of NPK fertilizer (2457 kgha^{-1}), a statistically very significantly higher soybean yield was achieved compared to the control variant of the experiment (2161 kgha^{-1}) and a statistically significantly higher yield compared to the application of AN nitrogen fertilizer (2356 kgha^{-1}) and water extract from plant material (2340 kgha^{-1}). A statistically very significantly higher yield was also recorded on the variants with the application of nitrogen fertilizer AN and water extract from plant material compared to the control variant of the experiment.

Conclusion

Based on the analysis of the two-year results of the influence of the time of basic tillage and fertilization on soybean yield, it can be concluded:

The highest yield of soybeans is achieved during the autumn basic tillage of the soil, and during the later basic tillage, the yields of soybeans decrease very significantly.

The application of NPK fertilizer achieves the highest soybean yield, while the foliar application of aqueous extract from plant material and nitrogen fertilizer AN contributes to a significant increase in soybean yield.

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PROMOTING SMART AGRICULTURAL PRACTICES IN MAIZE PRODUCTION INBIH - H2020 SMARTWATER PROJECT

Mihajlo Marković[1], Nataša Čereković[1], Đurađ Hajder[1], Milan Šipka[1],Nery Zapata[2], Teresa A. Paço[3], Erminio E. Riezzo[4], Sabrija Čadro[5], Mladen Todorović[6]

Abstract: Agricultural practices in Bosnia and Hercegovina demand different improvements, including smart management of land and water resources. A new H2020 project started in 2021 in this regard. The objective of this publication is to spread knowledge about SMARTWATER project by describing different achievements in two years of implementation (2021-2022), to invite target groups to participate in the action and to promote smart agricultural practices. Presented results indicate that the implementation is at a satisfactory level. Project consortium will continue with efforts, including twinning, networking, research, dissemination and increasing competency and fund rising skills.

Keywords: twinning, research, exchange, irrigation, smart technologies

Introduction and literature review

Smart management of land and water resources in Bosnia and Herzegovina (BiH), as a country with a good irrigation potential but rainfed agriculture practices, is very important (World Bank, 2012; FAO, 2017). In line with these a new H2020 project SMARTWATER[2] started with its implementation on the 1st of January 2021. The main research themes are in line with the needs of BiH agriculture and relevant research, and include: 1) cloud-based smart technologies (Romero et al., 2012; Todorovic et al., 2016), 2) new generation of satellite remote sensing data (Paço et al., 2014; Mateos et al., 2013), 3) water-energy-food nexus (Zapata et al., 2017) and 4) climate change impact to agriculture (Stričević et al., 2014; Todorovic et al., 2018). The effect of irrigation

[11] University of Banja Luka, Faculty of Agriculture, Bulevar v. Petra Bojovića 1A, Banja Luka, Bosnia and Herzegovina (mihajlo.markovic@agro.unibl.org); [2]Agencia Estatal Consejo Superior de Investigaciones Cientificas, Calle Serrano 117,Madrid,Spain; [3]Universidade de Lisboa, Instituto Superior de Agronomia, Tapada da Ajuda 1349-017,Lisbon, Portugal; [4]SYSMAN PROGETTI & SERVIZI SRL, Via G. Lorenzoni 19, Roma, Italy; [5] University of Sarajevo, Faculty of Agriculture and Food Science, Zmaja od Bosne 8, Sarajevo, Bosnia and Hercegovina; [6]Centro Internazionale di Altistudi Agronomici Mediterranei, Via Ceglie 9, Valenzano, Italy.

[2]http://www.smartwater-project.eu/

and fertilization treatments was studied in maize (Živanović et al., 2015; Kresović et al., 2016; Stričević et al., 2017; Dodig et al., 2021).

The main objective of SMARTWATER is to reinforce new networking, research and S&T cooperation capacities of the University of Banja Luka (UNI-BL), the University of Sarajevo (UNSA) and other connected national institutions, in the field of sustainable agricultural water management and to increase their competency and fund raising skills for a successful participation in the European Union Research Programs.[3]The project duration is 36 months. SMARTWATER consortium consists of six partners[4]: UNI-BL, CIHEAM-IAMB, CSIC, ISA, SYS and UNSA and implementation is planned through five Work Packages (WPs).

The objective of this paper is to spread knowledge about SMARTWATER project by describing achievements in two years of implementation (2021-2022), to invite all project target groups to participate in the action and to promote smart and sustainable practices in agriculture in Bosnia and Hercegovina.

Opening new horizons: the project implementation started in 2021

period January-June 2021

The H2020 SMARTWATER project started with its implementation on the 1st of January 2021. The first activity was kick-off meeting, held in a hybrid form (27.1.). The event gathered 36 participants and great outcomes were achieved.

The dissemination was done in order to spread knowledge about project and to gather and involve target groups. We established SMARTWATER project logo and official website, as well as social media profiles on *Facebook*, *Twitter*, *LinkedIn* and *YouTube*, published posts and news. The joint experimental studies (WP3) were prepared. We published the info for master course in Bari (Italy) at CIHAEM-IAMB (WP2).

The UNI-BL team participated in the REA Cluster Event[5](20.5.) and in symposium AgroReS 2021[6] (27-28.5.). The 1st stakeholders' meeting was

[3]European Commission (2020). Grant Agreement, project number 952396, SMARTWATER.

[4]UNI-BL (UNIVERZITET U BANJOJ LUCI), CIHEAM-IAMB (CENTRO INTERNAZIONALE DI ALTISTUDI AGRONOMICI MEDITERRANEI), CSIC (AGENCIA ESTATAL CONSEJO SUPERIOR DEINVESTIGACIONES CIENTIFICAS), ISA (INSTITUTO SUPERIOR DE AGRONOMIA), SYS (SYSMAN PROGETTI & SERVIZI SRL), UNSA (UNIVERZITET U SARAJEVU).

[5]https://faster-h2020.eu/wp-content/uploads/2021/05/Final-report-Cluster-meeting-Agri-Nat-Res.pdf

[6]https://agrores.net/en/

organized by UNSA (7.6.) and more than 30 participants joined. We also prepared and submitted to the European Commission (EC) project reports (deliverables) in WP1 and WP5.

period July-December 2021

The main project activity in this period was the 1st summer school. It was organized by UNI-BL in BiH in Trebinje (30.8. – 3.9.) and 40 participants joined. The topic was "*Integrated approach for agricultural water management*".

Project financial and administrative issues were discussed. We worked on the equipment procurement. Also, cultivations and plant and soil samplings in maize were done. SMARTWATER project was presented in an interview to INTRASOFT International[7]. We participated in the ERA *Info Day*[8] *event*(9.7.).We joined two conferences, *"Soils for future under global challenges"* organized in Serbia (21-24.9.) and *"Agrosym 2021"* organized in BiH (7-10.10.)[9].

As one of the main activities, ISA organized the first advanced training course in Portugalin Lisbon(27.9. – 1.10)and 32 people joined. The topic was "*Advanced remote sensing technologies and tools for crop water requirements estimates and irrigation scheduling*".

Master students started their course (1.10.). CATCHaCORN[10] students' competition was organized (24.10.). Dissemination activities were pronounced and SMARTWATER was further promoted. The 1st year of experiments was finished. The remaining project reports in 2021 were sent to EC.

Staying in lane: the project implementation continued in 2022

period January - June 2022

The second project year has started with a lot of experience behind us. We begin to prepare data for the 1st periodic report and to implement forthcoming activities. Dissemination activities continued. The 2nd year of experiments was prepared. We joined the *"World Water Day*[11]*"*in Patras in Greece (22.5.) and the"

[7]https://www.netcompany-intrasoft.com/

[8]https://research-and-innovation.ec.europa.eu/events/horizon-europe-info-days/era-and-widening/july-2021_en

[9]https://congress.sdpz.rs/ and http://agrosym.ues.rs.ba/

[10]http://www.smartwater-project.eu/the-first-competition-in-maize-harvesting-catchacorn-event/

[11]http://www.smartwater-project.eu/world-water-day-22-march-2022-patras-greece/

5th International Scientific Conference on Water[12]*"* in Szarvas in Hungary (22-24.3.).

We continued to cooperate with similar H2020 projects. We presented the project at *"AgroReS 2022*[13]*"* (26-28.5., Trebinje, BiH). ISA team joined the "ENCONTRO CIÊNCIA[14]" in Lisbon (Portugal) from 16th to 18th of May 2022.

UNSA team organized the second stakeholders' meeting (20.6.). The topic was "*Promotion of sustainable agricultural water managements practices in Bosnia and Herzegovina*" and 83 participants joined. ISA participated in the" *National Fair of Agriculture*" in Portugal (4-12.6.).Two students from BiH were awarded master's degree at CIHEAM-IAMB. We submitted reports in WP 1, WP 4 and WP 5.

period July- December 2022

The main project activity in this period was the second summer school. It was organized by UNSA in Sarajevo in BiH (18-22.7) with 46 participants. The topic was "*Smart technologies and best practices (technical and practical) for sustainable and environmentally efficient water management in agriculture*". SMARTWATER was presented at the "*International Symposium on Managing Land and Water for Climate-Smart Agriculture*[15]" held in Vienna in Austria (25-29.7.).Project reports in WP 2 and WP 5 were submitted. UNSA team organized the first workshop on funding opportunities and proposal drafting in Sarajevo in BiH (11-13.10.).We presented SMARTWATER at three conferences, the first one was held in Velke Bilovice[16]in Czech Republic (6-7.10.), the second one in Ohrid[17] in North Macedonia (12-14.10.) and the third one in Beja[18] in Portugal (18-20.10.).

As one of the main activities, CSIC organized the second advanced training course inZaragoza in Spain (26-30.9.)andmore than 20 people joined.The topic was "*Use of innovative technologies and tools for collective and on-demand pressurized irrigation systems*".

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[12]http://www.smartwater-project.eu/5th-international-scientific-conference-on-water/

[13]https://agrores.net/en/

[14]https://www.encontrociencia.pt/2022/

[15]http://www.smartwater-project.eu/smartwater-project-at-the-international-symposium-on-managing-land-and-water-for-climate-smart-agriculture/

[16]http://www.smartwater-project.eu/smartwater-project-at-the-international-conference-on-urban-water-2022/

[17] https://isaf2022.isaf.edu.mk/

[18]http://www.smartwater-project.eu/smartwater-project-at-ix-national-congress-on-irrigation-and-drainage-in-beja-portugal/

The second PSG_SEAB meeting was held by UNI-BL in Banja Luka and Teslić (BiH), from 13th to 16th of December 2022. This was great opportunity to gather all PSG and SEAB members, to have the overview of activities and achievements in two years of project implementation and to plan future activities.

At the end of 2022 we summarized our efforts, sent all the necessary project reports and established the framework for the activities in 2023. The effort of SMARTWATER project consortium in 2021 and 2022, when it comes to the pre-defined activities, is presented in Table 1.

Table 1. The overview of activities within SMARTWATER project in 2021 and 2022

no.	*Activity type*	*Accomplished*	*Project report reference*
1	advanced training courses	2/3	D2.4 / D5.9
2	summer schools	2/3	D2.3 / D5.8
3	joint research activities	2/3	D3.1 / D3.2 / D3.3
4	meetings – roundtable debates	2/3	D4.3
5	post-graduate MSc programs	3/3 (1st year)	D2.8 / D2.9 / D2.10
6	mutual staff exchanges	3/13	D3.1 / D3.2 / D3.3
7	hands-on workshops	1/3	D2.5
8	international conferences	13/3	-
9	smart water management tools	2/2	-

Slowing down: future plans and activities in 2023

In 2023, SMARTWATER project life will hopefully continue. The activities to be organized include remaining advanced courses, summer schools and exchanges, the last year of MSc and experimental studies, and the last year of stakeholders' meetings, hands-on workshops and participation to conferences.

Joint experimental studies – basic considerations

Within WP 3, the joint research includes experiments in 3 years and at two locations in BiH (Aleksandrovac and Butmir).The target plant species is maize (*Zea mays* L.), hybrid BL 43 (FAO 400 group). The RCB design include two factors, irrigation (3 irrigation levels) and fertilization (2 nitrogen levels).The aim of these experiments is to find the best combination of irrigation and fertilization treatments in maize production in agro-ecological conditions of Banja Luka and Sarajevo (BiH) and to promote smart agricultural practices in land and water management in agriculture in BiH.

Conclusion

In two years of SMARTWATER project implementation a lot of twinning activities were performed. We organized summer schools and advanced courses, participated in more than 15 conferences in BiH and abroad, joined 6 EC workshops, and had 2 stakeholders' meetings. Along with these, we have worked on project reports, dissemination, staff exchange and publications within the main research themes. Our mutual efforts contributed the increase of networking and research between UNI-BL, UNSA and connected institutions and the increase of our competency and fund rising skills for new proposals toward the EC, which are the main project objectives. The presence of a lot of activities in 2023 should not stop project consortium in putting more effort to achieve goals and to work on project legacy.

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CONTENT OF POLYPHENOL COMPOUNDS IN THE DRY MATTER OF ITALIAN RYEGRASS

Jordan Marković[1], Đorđe Lazarević[1], Vladimir Zornić[1]

Abstract: The aim of this study was to examine the influence of harvest time and nitrogen fertilizer application on the content of total polyphenols, flavonoids and condensed tannins in the dry matter of Italian ryegrass. The plants were harvest in 2017, in spring and early summer. The results obtained in this study showed that the contents of polyphenols, flavonoids and condensed tannins in the dry matter of Italian ryegrass (12.45 g / 100 g dry matter, 7.45 g / 100 g dry matter and 956.55 mg / 100 g dry matter, respectively) were higher when the plants harvested in early summer than in the spring. The application of nitrogen fertilizer did not have effect on the synthesis of polyphenolic compounds.

Keywords: Italian ryegrass, polyphenols, flavonoids, condensed tannins

Introduction

Italian ryegrass (*Lolium italicum syn. L. multiflorum*) is spread all over the World, but it is mostly grown in Europe and Asia. It is characterized by high yields and quality, and it is nutritive high valued forage species. It is primarily used for animal nutrition in the form of green forage or hay, and it can also be prepared as high-quality silage. Italian ryegrass is an important component in grass-legume mixtures, it combines particularly well with red clover (Knežević and Stanisavljević, 2018; Đorđević and Dinić, 2007).

Grasses have been extensively studied to determine effects of water soluble carbohydrates on ruminant performance (Cosgrove et al. 2015; Lee et al. 2006) and factors affecting the water soluble carbohydrate concentration, such as defoliation frequency (Loaiza et al. 2017; Turner et al. 2015), nitrogen application (Loaiza et al. 2017), and drought stress (Rigui et al. 2019) have also been studied. It seems possible that some of the positive effects of Italian ryegrass on ruminant performance may be due not only to high digestibility and high carbohydrate concentration, but also to benefits provided by molecules such as phenolic compound. However, relatively little is known

[1]Institute for forage crops Kruševac, 37251 Globoder, Kruševac, Serbia (jordan.markovic@ikbks.com)

about the soluble phenolic compound composition of Italian ryegrass, making it difficult to determine which compounds might positively influence animal performance. Among the numerous benefits for ruminant nutrition, it is important to mention that some forage species may contain compounds that can reduce protein degradability. There is an increased interest in studying those forage species that reduce proteolytic processes during ensiling and reduce protein degradation in the rumen. Forage species that contain protein and tannin complexes, such as birdsfoot trefoil, have the property that their protein are less degradable, compared to that do not contain tannins, such as alfalfa (Julier et al. 2003). Some works have been published on the mechanism of activity of those compounds and a positive effect they have on yield and quality of various agricultural crops (Agnieszka and Grazyna, 2021; Di Mola et al. 2019; Roupheal et al. 2018). Attention should be paid to the fact that crop productionshould provide high yields of good quality (Kagan, 2021), free of genetic modification, which is particular importance in the ra of unfavourable environmental conditions and global loss of plaughed land.

The aim of this investigation was to determine the content of polyphenolic compounds – Total polyphenols, flavonoids and condensed tannins in the dry matter of Italyan ryegrass harvested in the spring and early summer depending on the different rate of nitrogen fertilizer.

Materials and methods

The Italian ryegrass was sown in 2016 at the experimenta field of Institute for forage crops Kruševac. The trial was established by the method of randomized complete block design in three replications as a two factorial (harvest time x nitrogen fertilizer application). The plants were harvested two times in 2017: in the spring – 09.05. and in the early summer – 22.06. Nitrogen fertilizer was applied at rate 60 kg, 90 kg and 0 as control.

The sampled plant material was prepared for the drying process in laboratory conditions. The plant material was dried in a thin layer in a drafty and dark place where the temperature ranged from 18-22° C, and the air humidity ranged from 55-65%. The drying process was regularly controlled, where damaged specimens and those that had changed color were removed. After a 7-day drying process, the dry plant material was crushed and packed in dark glass containers until the extraction process.

About 2.0000 g of dried homogenized plant material was weighed and 20 ml of methanol : water : HCl (80 : 19 : 1) extraction solution was added. After

standing for 2 hours at room temperature, the solution was decanted, and the solid residue was extracted 2 more times in the manner already described (20 ml and 10 ml of the extraction solution). The filtrate obtained by squeezing the collected extracts through a Bichner funnel was transferred to a normal 50 ml vessel, and diluted with solvent to the line. The obtained extract was stored in a dark and cold place.

The determination of total polyphenols of Italian ryegrass extracts was performed with the Folin-Ciocalteu reagent according to the method of Singleton and Rossi (1965) with some modifications. The absorbance is measured at 765 nm by UV-Vis spectrophotometer (HALO RB-10, Dynamica). The results are expressed in g gallic acid equivalent / 100 g plant dry matter by reference to the calibration curve of gallic acid. The total flavonoids determination of Italian ryegrass extracts was performed according to the method described by Jia et al. (1999) with some modifications. The absorbance is measured at 415 nm using a UV-Vis spectrophotometer (HALO RB-10, Dynamica). The results are expressed in g quercetin equivalent / 100 g plant dry matter with reference to the quercetin calibration curve. Condensed tannins content of Italian ryegrass extracts were determined using the vanillin assay described by Makkar and Becker (1993) with some modifications. The absorbance at 550 nm was measured against a blank using a UV-Vis spectrophotometer (HALO RB-10, Dynamica). The total concentration of condensed tannins was expressed in mg of catechin equivalents / 100 g plant dry matter with reference to the catechin calibration curve.

Data were analyzed using ANOVA in a randomized block design using the Stat. Soft. STATISTICA 6. The statistical significance of differences tested using Tukey – test, and significant differences among means were accepted at $p < 0.05$.

Results and discussion

Results obtained in this investigation for total polyphenols, flavonoids and condensed tannins in the dry matter of Italian ryegrass are presented in the Table 1.

The results of this study showed that the content of total polyphenols in the dry matter of Italian ryegrass was higher in plant material harvested in early summer (12.45 gE gallic acid / 100 g dry matter) compared to plant material harvested in the spring (10.05 gE gallic acid / 100 g dry matter). The highest content of total polyphenols was found in the treatment without fertilization, whereas the lowest content was found in the treatment with the highest amount of applied nitrogen mineral fertilizer. Such results were expected, since this

group of compounds do not contain large amounts of nitrogen, so the application of nitrogen fertilizers did not significantly affect their biosynthesis.

Table 1. Content of total polyphenols, flavonoids and condensed tannins in dry matter of Italian ryegrass

Applied Nitrogen fertilizer	Total polyphenols gE gallic acid / 100 g dry matter		Flavonoids gE quercetin / 100 g dry matter		Condensed tannins mgE catechin / 100 g dry matter	
	Spring	Erly Summer	Spring	Erly Summer	Spring	Erly Summer
0 kg	10.05^{b}	12.45^{a}	7.24^{a}	7.48^{a}	464.89^{d}	956.55^{a}
60 kg	9.72^{b}	10.15^{b}	4.75^{c}	5.81^{b}	452.79^{d}	624.87^{b}
90 kg	7.79^{c}	8.16^{c}	4.14^{d}	4.07^{d}	530.03^{c}	632.15^{b}
Average	9.18^{B}	10.25^{A}	5.37	5.78	482.57^{B}	737.85^{A}
Total average	9.72		5.58		610.21	

Different small letters denote significan means between treatments, $p < 0.05$
Different large letters denote significant means between treatments, $p < 0.05$

A similar tendency was established for the content of flavonoids, which was 7.24 gE quercetin / 100 g dry matter in plant material of Italian ryegrass harvested in spring and 7.48 gE quercetin / 100 g dry matter in plant material harvested in early summer grown without nitrogen fertilizer application. The influence of nitrogen mineral fertilizer application had a different effect on the content of condensed tannins (Table 1). The lowest content of condensed tannins was found in the treatment with 60 kg of nitrogen fertilizer application (452.79 mgE catechin / 100 g dry matter of Italian ryegrass harvested in spring and 624.87 mgE catechin / 100 g dry matter of Italian ryegrass harvested in early summer). We assume that higher values for the content of total polyphenols, flavonoids and condensed tannins in the dry matter of Italian ryegrass harvested in the aearly summer were due to higher temperatures, longer days and a greater number of sunny hours during the days. Kagan (2021) also indicated that concentration and composition of soluble phenolic compounds in the plants are affected by sample preparation, presence of pathogens or endophytes, environmental stresses, time of day, plant maturity and genetic background. In accordance with finding by Choi et al. (2017) we could suggest that Italian ryegrass silage could potentially be the source as functional diet. On the other hand, little is known regarding the compounds responsible for its antioxidant and anti-inflamatory activities.

Conclusion

In conclusions, phytochemical composition and concentration vary among forages, with polyphenols comprising the largest group and having been associated with many health properties. The results obtained in this research showed that the highest content of polyphenols, flavonoids and condensed tannins was found in the second cut – in early summer. Such results can be associated with longer days, higher temperatures and longer sunny intervals in this period of study. Further investigations are necessary to determine the impact of all these factors. The application of nitrogen mineral fertilizer showed no influence on the synthesis of polyphenolic compounds.

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ANALYSIS OF GENOTYPE BY ENVIRONMENT INTERACTION FOR SPIKE TRAITS IN WINTER SIX-ROW BARLEY

Kamenko Bratković[1], Kristina Luković[1], Vladimir Perišić[1], Jelena Maksimović[2], Jasna Savić[3], Vera Đekić[4], Mirela Matković Stojšin[5]

Abstract: This research was conducted with some spike traits of twenty winter six-row barley genotypes in six environments. The aim of this study was to determine the significance and take advantage useful genotype by environment interacton (GEI) by applying AMMI-1 model. High statistical significance GEI was determined. Wide adaptability genotypes were J-29, J-33, J-9 and J-21 for spike length (SL) as Grand and Ozren for grain number per spike (GNS). The winner genotypes in all environments were Ozren and Grand for SL as Ozren for GNS. All the examined environments can be considered as one megaenvironment, which indicates that unpredictable interactions dominate in this research.

Keywords: barley, spike traits, GE interaction, AMMI model, stability

Introduction

Barley (*Hordeum vulgare* L.) is one of the most important cereal crops in the world. Based on number of grains row per spike there are two different forms of barley spike. Six-row spikes show fertile lateral spikelets compared with two-row spikes with sterile lateral spikelets (Ullrich, 2011). Spike length and grain number per spike are one of the most important components of grain yield of barley. Six-row barley per unit of spike length contains a greater number of grains compared to two-row barley, so even a small increase in spike length is followed by a significant increase in the grain number per spike. That is why increasing the spike length of the six-row form of barley is one of the main goals in breeding (Dodig, 2000). An increase in grain number per spike affects

[1]Center for small grains and rural development, Save Kovaševića 31, 34000 Kragujevac, Serbia (kamenko@kg.ac.rs)
[2]Institute for soil science, Teodora Drajzera 7, 11000 Beograd, Serbia
[3]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Zemun, Serbia
[4]Univrsity of Niš, Faculty of Agriculture, Kosančićeva 4, 37000 Kruševac, Serbia
[5] Institute Tamiš, Novoseljanski put 33, 26000 Pančevo, Serbia

the increase in grain yield, and its increase can compensate for the reduced number of spikes and plants per unit area (Barczak and Majcherczak, 2009).

Genotypes have different expression depending on environmental conditions (Bocianowski et al., 2019). In this way, they form genotype by environment interaction (GEI) whose presence complicates the effectiveness of selection (Pržulj et al., 2015). A special group of models for analysis of GEI are linear-bilinear statistical models that have the ability to model complex interactions in multiple dimensions. The additive main effects and multiplicative interaction (AMMI) analysis are widely used (Alake and Ariyo, 2012).

Materials and methods

The data set in this paper represents spike traits as spike length and grain number per spike of 20 winter barley genotypes, 11 recognized cultivars and 9 advanced breeding lines of F7 and F8 generation (marked with J). Genotypes were origin from Republic of Serbia and according to type of spike belong to six-row barley (*Hordeum sativum, ssp. vulgare).*

Field trials were conducted over a two growing seasons (2008/2009 and 2009/2010) at three locations in Serbia under dry farming conditions: Kragujevac (KG)-central Serbia (44º02'N 20º56'E, altitude 185 m, Smonitza type soil), Zemun Polje (ZP)-north Serbia (44º49'N, 20º17'E, altitude 96 m, calcareous Chernozem) and Zaječar (ZA)-eastern Serbia (43º53'N, 22º17'E, altitude 144 m, non-carbonate Smonitza). The first season was moderately humid (based on precipitation) with warm and sunny spring (stem elongation, anthesis and grain filling stage) while the second season was humid with colder and cloudly spring. Combinations year and locality there were six environments were labeled as follows: KG09, ZP09, ZA09 represent locations KG, ZP and ZA in the first growing season, while KG10, ZP10 and ZA10 represent the same locations in the second growing season, respectively. The experiments were set up according to Fisher's plan of randomized blocks with four replications and 5 m^2 plots. Sowing was machine in mid-October. At full maturity, from each plot 20 primary spikes were analyzed according the length (cm) and number of grains.

Spike traits data were analyzed using linear mixed model with homogeneous residual error variances. The choice between models is based on the value of the Akaike Information Criterion (AIC). The interaction and assessment of genotype stability in different environmental conditions was analyzed using a linear-bilinear model Additive Main effects and Multiplicative

Interaction (Gauch and Zobel, 1996). The statistical significance of individual AMMI models was tested, and the AMMI-1 view was applied, taking into account all the advantages of the applied approach (Yan and Tinker, 2006). In the biplot graph, AMMI parameters on the ordinate are the values of the interaction principal components (IPC-1), while on the abscissa are the mean values of the genotypes and the environments. AMMI-1 model had its estimated value for interaction and equal IPC-1g x IPC-1e. This estimated interaction is part the AMMI-1 expected values of traits for any genotype and environment combination. The rest is the additive AMMI-0 part of the AMMI model is simply the genotype average plus the environment average minus the general average (Zobel et al., 1988). Statistical data processing was performed using R software, version 3.1.2 (R Development Core Team, 2014).

Results and discussion

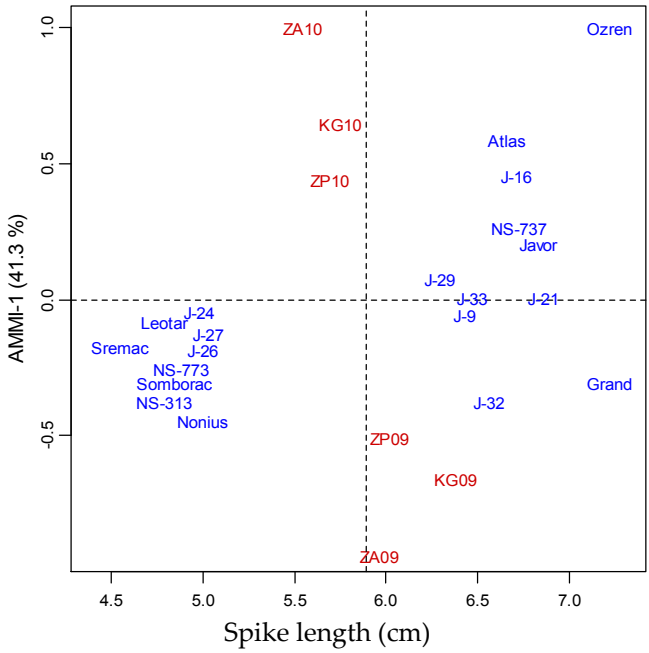
To explain the variation for spike length (SL) and grain number per spike (GNS), using Akaike's information criterion (–583.6, 1391.8, respectively), it was determined that the most suitable mixed model with homogeneous error variances environments (Table 1). For both traits, the effect of genotype and the interaction of genotype with the external environment stand out as a highly significant factor (P < 0.01) for explaining the variation, while the environment in which the research was conducted did not show significance (P > 0.05). Therefore, the application of the AMMI model is justified because, based on Shaft and Price (1998), this model has an advantage in a situation of significant interaction and nonsignificant main effects.

Table 1. Mixed model with homogeneous variances of environmental errors of spike length (SL) and grain number per spike (GNS) of twenty six-row barley across six environments

Source variation	SL		GNS	
	Fixed effect			
	F	*P*	*F*	*P*
Genotype (G)	63.35	<0.0001	12.66	<0.0001
	Random effect			
	Z	*P*	*Z*	*P*
Environment(E)	1.50	0.0670	1.57	0.0588
Interaction(G×E)	6.79	<0.0001	6.80	<.0001
Residual σ^2	13.08	<0.0001	13.08	<.0001

P < 0.01 – highly significant; *P* < 0.05 – significant; *P* > 0.05 not significant

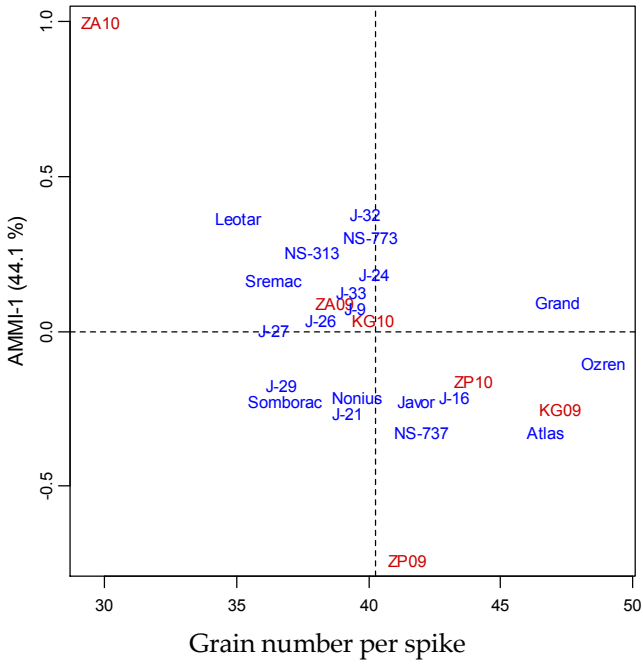
AMMI-1 analysis for SL of six-row barley showed that the first principal component explained 41.3 % of the genotype by environment interaction (Graph 1). Regarding the SL low interaction values indicating a high stability were observed for genotypes J-24, Leotar and J-27 with below average of spike length (5.89 cm). In case breeding lines J-29, J-33, J-9, J-21 the highest stability was associated with high values of this trait. These genotypes maintain the yield level in all environments and are less sensitive to changes in external conditions. The stability of certain above average genotypes can generally be considered as the ability to be well adapted to unfavorable climate conditions (Ciulca et al., 2018). Elakhdar et al. (2017) such genotypes are considered widely adaptable and important in the use of beneficial interaction effects. Genotype Ozren was the lowest stability and the highest values of lenght which in accordance with Mohhamed et al. (2009) which indicate that it is more difficult to achieve stability at high values of properties.



Graph 1. AMMI1 biplot for spike length of twenty six-row barley genotypes across six environments

Based on the results of the AMMI-1 model for the GNS of six-row barley (Graph 2), we observe that the first principal component explained 44.1 % of the sum of squares of the interaction between genotype and external environment.

The highest stability was recorded by genotypes J-27, J-26, J-9, J-33, Grand and Ozren which showed high variaton of this trait over the environments. Regarding wide adaptation, genotypes Grand and Ozren stood out. They were very stable and with a significantly higher number of grains compared to the general average (40.2). None of the genotypes showed significant unstability. For the GNS, higher values of the trait were not associated with unstability as for the SL. Only genotypes J-33 and J-9 showed stability in both traits, while Ozren was the most unstable in SL and among the most stable in GNS.



Graph 2. AMMI1 biplot for grain number per spike of twenty six-row barley genotypes across six environments

AMMI model provides an agronomically meaningful interpretation of the data which is usually desirable in order to make reliable traits estimations. They are given in Table 2 AMMI-1 estimates for SL and GNS. These estimated values are used for prediction and recommendation, and their values depend on the characteristics of the investigated environments. In such cases when the environments are location-year combinations, Zobel et al. (1988) points out that a locality suitable for prediction and recommendation is one whose interaction effects differ slightly from year to year. The environments of each locality in SL differ significantly in the interaction effect. Gauch and Zobel (1997) suggest yes

large unpredictable interactions and diference between years require other access so the best predictive strategy is not to try to exploit interactions (only AMMI-0). For the GNS, this approach was not applied.

It is noticed that they were Grand and Ozren in SL as Ozren in GNS winner and recommended genotypes in all environments (Table 2). AMMI-1 model data compared to unadjusted data in our research (results not shown) has a smaller number of winning genotypes. We had three winners for both traits (Grand, Ozren and NS-737 for SL; Grand, Ozren and Atlas for GNS). This is in agreement with the results obtained by Egesi et al. (2002) indicating that AMMI-1 model ignoring irrelevant interaction noise and error thus reducing the number of winner genotypes. These were also the genotypes with the highest average trait values in our research because AMMI-1 estimates interaction has low values (GNS) or is ignored in case of differences in effects of environments (SL), so values estimates were based mainly on the main effect (AMMI-0).

Table 2. AMMI-1 estimated values for SL (cm) and GNS (in brackets) twenty six-row barley genotypes across six environments

Genotypes	KG09	ZP09	ZA09	KG10	ZP10	ZA10
Grand	**7.71**(54.2)	**7.35**(48.5)	**7.29**(45.7)	**7.08**(47.2)	**7.02**(51.0)	**6.88**(37.0)
NS-313	5.28(44.8)	4.92(39.0)	4.86(36.3)	4.65(37.8)	4.59(41.6)	4.45(27.7)
Ozren	**7.71(55.9)**	**7.35(50.3)**	**7.29(47.4)**	**7.08(48.9)**	**7.02(52.7)**	**6.88(38.5)**
Sombor	5.34(43.8)	4.98(38.2)	4.92(35.3)	4.71(36.8)	4.65(40.6)	4.51(26.4)
Sremac	5.04(43.4)	4.68(37.8)	4.62(34.9)	4.41(36.4)	4.35(40.2)	4.21(26.2)
Atlas	7.16(53.8)	6.80(48.2)	6.74(45.2)	6.53(46.7)	6.47(50.5)	6.33(36.2)
Leotar	5.28(42.0)	4.92(36.7)	4.86(33.6)	4.65(35.1)	4.59(38.9)	4.45(24.5)
NS-773	5.37(47.0)	5.01(41.2)	4.95(38.6)	4.74(40.1)	4.68(43.9)	4.54(30.0)
Nonius	5.49(46.4)	5.13(41.0)	5.07(38.1)	4.86(39.6)	4.80(43.4)	4.66(29.1)
NS-737	7.21(49.1)	6.85(43.5)	6.79(40.5)	6.58(42.0)	6.52(45.8)	6.38(31.5)
Javor	7.32(48.8)	6.96(43.2)	6.90(40.3)	6.69(41.8)	6.63(45.6)	6.49(31.3)
J-26	5.49(45.2)	5.13(39.5)	5.07(36.7)	4.86(38.2)	4.80(42.0)	4.66(27.9)
J-32	7.05(46.8)	6.69(41.0)	6.63(38.4)	6.42(39.9)	6.36(43.7)	6.22(29.9)
J-24	5.47(47.2)	5.11(41.4)	5.05(38.7)	4.84(40.2)	4.78(44.0)	4.64(30.2)
J-9	6.92(46.5)	6.56(40.8)	6.50(38.0)	6.29(39.5)	6.23(43.3)	6.09(29.2)
J-33	6.96(46.3)	6.60(40.5)	6.54(37.8)	6.33(39.3)	6.27(43.1)	6.13(29.1)
J-27	5.52(43.4)	5.16(37.7)	5.10(34.9)	4.89(36.4)	4.83(40.2)	4.69(26.1)
J-29	6.78(43.7)	6.42(38.1)	6.36(35.2)	6.15(36.7)	6.09(40.5)	5.95(26.2)
J-16	7.20(50.2)	6.84(44.6)	6.78(41.7)	6.57(43.2)	6.51(47.0)	6.37(32.8)
J-21	7.35(46.3)	7.48(40.6)	6.93(37.7)	6.72(39.2)	6.66(43.0)	6.52(28.7)

Since the existence of one winner genotype is observed in all localities, which is according to the winner-method proposed by Gauch (2013) za AMMI-1 model, the data suggest considering all environments as a single mega-environment for spike traits of barley. Our trial is clearly dominated by unpredictable interactions over predictable ones which acording Gauch and Zobel (1997) indicates that they are typically associated with years and make mega-environments less numerous and less advantage can be taken of specific adaptations. This is the main reason for the existence of only one such environment in this paper and the reason why specific adaptations do not significant.

Conclusion

Genotype by environment interaction (GEI) is complex problem which complicates the selection process field crops. Our study showed complex and importance investigate of interaction. Variable climatic conditions require barley breeders to select adaptability genotypes and therefore the AMMI model is very suitable. Only genotypes J-33 and J-9 showed stability in both traits while stability not associated with average values of genotypes. Genotypes with wide adaptation and suitable for growing in different agroecological conditions were J-29, J-33, J-9 and J-21 for spike length (SL) as Grand and Ozren for grains number per spike (GNS). However, based AMMI-1 estimated values, the winner genotypes in all environments were Ozren and Grand for SL as Ozren for GNS. This genotypes had the highest values of spike traits. Therefore all the examined environments can be considered as one megaenvironment, which indicates that unpredictable interactions dominate in this research, due to which specific adaptations were not of high importance. All this information could be use in barley breeding in order to increase the genetic gain for grain yield.

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INFLUENCE OF HEAVY METALS ON WHEAT STEM DEVELOPMENT

Violeta Mickovski Stefanović[1], Dragana Stanisavljević[2], Jasmina Bačić[1], Predrag Brković[1], Miloš Pavlović[1], Mirela Matković- Stojšin[1]

Abstract: Plants that grow in contaminated soil absorb and accumulate heavy metals in its above ground organs. Research was conducted in which content of heavy metals in wheat stem in phenophases of tillering and beginning of stemgrowth, and also stem hight of wheat from soil level to tip of appical leaf. The aim of research was to determine the influence of heavy metals on first stages of wheat varieties *Pobeda* and *Ljiljana.* Trial was conducted in pots with controled conditions of heat and moisture with adding different concentrations of heavy metals mixture. Higher concentrations of heavy metals had significant effect reducing the growht and wheat stem development of both wheat varieties. Significant concentration of heavy metals was detected throug analisis in stems of wheat.

Key words: atomic adsorbent spectrophotometry, heavy metals, wheat stem, *Triticum sp*

Introduction

Certain number of heavy metals (iron, manganese, copper) are necessary in small amounts for growth and development of plants. These metals are microelements essential for plant development, but in higher concentrations they can be toxic. Some heavy metals belong to the group of functional metals, and their presence in vegetative and generative organs of plants is not toxic. Selenium, cobalt and silicium belong to group of functional heavy metals. Other heavy metals are toxic for plants, but also for animals and humans which use plants for in their diet. Understanding of factors that influence bioavailability and behavior of heavy metals in soil is necessary. In recent times concentration of zink in some soils is getting higher, especially in countries with developed

[1]Tamiš Research and Development Institute, Novoseljanski put 33, Pančevo, Serbia (mickovski.stefanovic@institut-tamis.rs)
[2] Toplica Academy of Vocational Studies-Department of Agricultural and Food Studies, Ćirilo i Metodije 1, Prokuplje, Serbia

industry that do not have adequate measures for protection of environment. For zinc accumulation dinamics the most important factors are pH value, $CaCO_3$ content in soil and mechanical structure of soil. Plants that grow on soil contaminated with heavy metals assimilate and accumulate them in their above ground organs (Jakovljević et al., 1997). Presence of mining sludge causes imobilisation of metals in soil becouse adding ferous oxides in soil adsorption of Pb and Zn occurs. Adding of manure, enhancing of microbial breathing and production of CO_2 triggers drop of pH and desorpcion of Pb and Zn (Mertz, 2022).

Rapid increase of human population on Earth dictates increase in need for more food. However, more and more heavy metals released in environment by antropogenic factors can decrease yeald and quality of products in Agriculture (Popović-Vukeljić, 2002). Heavy metals pose serious threat to human health when they are found in food. In agricultural products it is required that content of heavy metal is known. In research (Rajković et.al. 2014) analisis of regresion for anticipation of Cd and Pb in wheat and contribution to uncertainty in those predictions, in relation to entrances to regresion model was examined. For each part 500 m x 500 m of soil which is agricultural soil in Nederland a thousand samples was examined, and varibles were pH of soil, organic compounds in soil, and concentration of heavy metals in soil. When compering to EU standards, content of lead and cadmium was higher than highest allowed concentration in soil. Uncertainty levels for regresion anticipation were 36% for cadmium and 52% for lead (Brus and Jansen, 2004). Air in industrial zones, also is significant factor in soil polution (Škrbić et al., 2004). Higher contetnt of lead can be also consecquence of some local spillage of lead salts as waste on small surface. Increased content of lead in soil can also happen due to it´s deposition from air contaminated from industry and vehicles (Rajković et al., 2014). Current agricultural practises include wide use of pesticides and mineral fertilisers, which are known to have negative impact on human health and degradating afects on environment (Rastija, 2022). Presence of heavy metals in soil, Cd, As and Pb was confirmed in researches that included fertilising of soil number of years with nitrogen mineral fertilisers, furthermore, on some locations in significantly high concentrations. Nitrogen fertilisers, as potentialcsource of heavy metals, can also have a signiticant affect on pollution of water (leaching and eutrofication), soil (souring) and air (denitrification and volatisation) (Kolar, 2022).

Plants absorb harmfull metals from soil through root, but can also in certain conditions absorb metals through leaves and branches. These harmful elements

are acumulated ususaly in above ground parts of plants. This is the main reason why is understanding of accumulation mechanisms, distribution and heavy metals metabolism of great ecological, scientific and practical significance (Rajković et al., 2012).

Inhibition of any component of photosyntetic apparatus will always have negative effect on phisiological activities, and as consequence it will have negative effect on plant growth. Higher concentrations of toxic metals have influence on parameters that show capacity for gases exchange during photosyntesis, such es stome conductivity (Li at al., 2013).

Procentage of absorbed heavy metals through leaves, compared to total absorpcion from one plant, depends of chemical properties of soil. (Youseff and Chino, 1991) highlight that the intencity of heavy metal absorption is dramaticaly decreased in soils that have pH 7 and higher.

On cellular level, conceqences of prolonged exposure to higher levels of toxic metals can be desintegration of membranes, loss of ions, peroxidation of lipides, degradation DNA/RNA and finaly cell death. As a prerequisite for normal plant growth and development, plants must maintain concentration of essential elements inside values that are considered normal-homeostasis (Stojanović, 2017).

Under the influence of higher contrentrations of lead in plants are inhibited main processes, such as germination, growth and development, photosintetis proces, wather intake, mineral diet and ensimatic activity (Agami and Mohamed, 2013; He at al., 2016; Sharma and Dubey, 2005).

Materials and method

Experiment to determine influence of heavy metals mixture on accumulation dynamics in wheat stem and wheat growth was conducted in greenhouse on Faculty of Agriculture, Belgrade-Zemun, where condition of air mosture and heath were controlled. The experiment was conducted in three repetitions, with total of 36 pots in which two wheat varieties *Pobeda* and *Ljiljana* were sown. Before sowing, pots were filled with 2 kg of dry extract *Novobalt* which was than contaminated with mixture of chemical compounds of heavy metals in form of solutions: zinc in form of zinc-acetate $Zn(CH_3COO)_2 \times 2H_2O$, lead in form of lead-acetate $C_4H_6O_4Pb \times 3H_2O$, chromium in form of chromium-threoxide CrO_3, copper in form of copper-sulfate $CuSO_4$ and cadmium in form of cadmium-nitrate $Cd(NO_3)_2 \times 4H_2O$.
Next doses of solutions were applied:

0 ppm (control), 100 ppm, 250 ppm

The content of heavy metals in wheat stem was measured parameter in this experiment, and also stem hight of individual wheat plants from ground level.

Wheat varieties *Pobeda* and *Ljiljana* were chosen for this experiment because they were most sovn domestic varieties in South Banat. In each pot 12 seeds were sown at depth of 5 cm. Samples from each repetition were analised in phases of tillering and stalk growth when four plants from each pot were collected. After this each individual plant was separated from root manually. Remaining herbal mass (above ground part of wheat plants) was washed with destiled water and soaked several hours in 0,1M HCl, with te purpouse of removing any residual soil and mineral oxides from herbal mass.

Next step was drying cleaned material in heat oven on 80°C and ground up. One gram of previously dried and ground sample was measured an 20 ml of 60% HNO_3 was added. This mixture was slowly boiled for two hours. After that, when mixture cooled, 3 ml of H_2O_2 was added, and mixture was boiled agen for 15 minutes. This last step with hidrogen-peroxide was repeated one more time. When mixture cooled of again, 5 ml of 5M $HClO_4$ was added and slow cooking was performed until white steam of perchloric acids could be seen (Jones and Case, 1990). After cooling, 5 ml of 5M HCl was added and than samples were quantitativly transferred in normal dishes 50 ml volume. These dishes were filled to it´s max volume with destilled water. Analysis of prepared samples was done by atomic absorbent spectrophotometry (Varian Spectr AA 220FS apparatus) with acetylene/air flame. Data from spectrophotometry was analysed with statistical package STATISTICA 8 for Windows and SPSS SPSS Statistics 17.0.

Results and disscusion

Wheat stem hight

Variety *Pobeda* had shorter stem, on average (38.70 cm) when comparing with variety *Ljiljana* (44.50 cm). Average stem hight varied form 34.00 to 42.60 cm in repetiotions for variety *Pobeda*, and 35.50 to 50.00 cm in repetitions for variety *Ljiljana* (tables 1 and 2).

Table 1: Stem height, variety *Pobeda*, cm

Variant	Zn, Pb, Cr, Cu, Cd	LSD 5%	LSD 1%
Control	42.60	7.9889	14.6647
100 ppm	39.50	11.4729	21.06
250 ppm	34.00	1.8371	3.3723
Average	38.70		

Table 2: Stem hight, variety *Ljiljana*, cm

Variant	Zn, Pb, Cr, Cu, Cd	LSD 5%	LSD 1%
Control	50.00	1.8371	3.3723
100 ppm	48.00	1.8371	3.3723
250 ppm	35.50	5.1143	9.3881
Average	44.50		

When soil was contaminated with 100 ppm heavy metals mixture, stem hight of variety *Ljiljana* grown in those pots was 48.00 cm, while variety *Pobeda* had shorter stem, 39.50 cm. When soil was contaminated with 250 ppm heavy metals mixture, stem hight of wheat variety *Ljilana* was 35.50 cm, and *Pobeda* 34.00 cm.

Photosintesis proces is most sensitive to toxic effects of lead, longterm exposure causes inhibition of chlorophyll biosintesis (Ernst at al., 2000; He at al., 2014). Researches have shown that lead has negative influence on photosinthesis, traspiration and stome conductivity when it´s concentration in soil is higher then 300 ppm (Fu and Wang, 2015). Differences in effects caused by stress from high concetrations of toxic metals show that different tipes plants have different responces (Zhao et al., 2017).

Heavy metal content in wheat stem

Heavy metals content varied greatly in both wheat varieties, *Pobeda* and *Ljiljana* (tables 3 and 4).

Ranking heavy metals by their average content in wheat stem of variety *Pobeda*, they are as follows:

- Zink>cadmium>copper>lead>chromium

Average lead content in wheat variety *Pobeda* was 3.77 mg kg^{-1} and varied from 2.46 mg kg^{-1} (control) to 5.45 mg kg^{-1} (250 ppm variant).

Ranking heavy metals by their average content in wheat stem of variety *Ljiljana*, they are as follows:

- Zink>cadmium>copper>lead>chromium

Average lead content in wheat variety *Ljiljana* was 3.08 mg kg^{-1} and varied from 2.14 mg kg^{-1} (control) to 4.33 mg kg^{-1} (250 ppm variant).

Results of statistical data analysis (analysis of variance) show that experimental factor heavy metlas mixture concentration for soil contamination, had significant effect on content of heavy metals in above ground parts of wheat, what can be clearly seen from calculated F-values.

Table 3: Content of heavy metals in the stem of wheat, variety Pobeda, mg kg-1

Variant	Zn	Pb	Cr	Cu	Cd	Ftest	LSD 5%	LSD 1%
Control	19.39	2.46	1.23	4.49	0.65	38832.27**	0.1197	0.1655
100 ppm	39.46	3.40	0.99	5.26	8.45	1094.38**	0.4569	0.6318
250 ppm	49.10	5.45	1.75	5.54	9.12	49105.52**	0.2678	0.3703
Average	35.98	3.77	1.32	5.10	6.07	-	-	-

Table 4: Content of heavy metals in the stem of wheat, variety Ljiljana, mg kg-1

Variant	Zn	Pb	Cr	Cu	Cd	Ftest	LSD 5%	LSD 1%
Control	29.99	2.14	0.58	4.65	0.39	38832.27**	0.1197	0.1655
100 ppm	77.67	2.76	0.58	5.36	13.46	62999.44**	0.3917	0.5417
250 ppm	79.13	4.33	1.23	5.41	17.31	58501.43**	0.4087	0.5652
Average	62.26	3.08	0.80	5.14	10.39	-	-	-

Conclusion

Vegetation experiments in vessels were performed with different concentrations of heavy metal mixtures. Higher concentrations of heavy metal mixture had negative effect on wheat stem hight. Higher concentrations of heavy metals significantly reduced plant growth in both cultivars.

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THE INFLUENCE OF HEAVY METALS ON THE DEVELOPMENT OF THE SURFACE OF WHEAT LEAVES

Violeta Mickovski Stefanović[1], Predrag Brković,[1] Svetlana Roljević Nikolić[1], Helena Majstorović[1], Dragana Stanisavljević[2], Predrag Ilić[3]

Abstract: Heavy metals represent a very significant group of environmental pollutants because they are potential metabolic inhibitors. Therefore, a study was conducted where the height of the wheat stem, as well as the surface of the leaves in the budding and leafing stages, were examined when contaminated with a mixture of heavy metals in concentrations of 250 and 500 ppm. The aim of the research was to determine the influence of heavy metals on the initial phenophase of wheat varieties *Pobeda* and *Ljiljana*. An experiment was carried out in volumetric flasks where two different concentrations of a mixture of heavy metals 250 ppm and 500 ppm were added under controlled conditions.

Keywords: atomic absorption spectrophotometry, heavy metals, leaf, *Triticum sp*

Introduction

Interest in the use of bioindicators as a means of monitoring and assessing environmental pollution with toxic metals is constantly increasing. Heavy metals reach the environment from natural sources, but also through anthropogenic activities, and once they reach the environment they do not disappear but accumulate in the soil, sediment, and biota and are increasingly becoming a growing global problem (Stanković, 2015).

Toxic metals come from contaminated air and soil. Toxic metals can be found in the soil as a result of their presence in the native rocks. Also, the irrational use of organic and mineral fertilizers can lead to soil contamination. Toxic metals are absorbed by the plant through its roots from the soil, and from the atmosphere through its leaves (Mickovski-Stefanovć et al., 2012).

[1]Tamiš Research and Development Institute, Novoseljanski put 33, Pančevo, Serbia (mickovski.stefanovic@institut-tamis.rs)
[2]Toplica Academy of Vocational Studies-Department of Agricultural and Food Studies, Ćirilo i Metodije 1, Prokuplje, Serbia
[3]Faculty of Philosophy, Ćirila i Metodija 2, Niš, Serbia

The constant increase of the human population, and the construction of large cities, industrial facilities, and roads significantly reduce agricultural areas, but also with various harmful agents.

As these areas are closer to the main polluters of the ecosystem, the effects on cultivated plants become more pronounced. The biggest polluters of agricultural land and crops are heavy metals. Several heavy metals (Fe, Mn, Cu, Zn, and Co) in small amounts are necessary for the growth and development of plants, but they are toxic to plants in high concentrations. Heavy metals come from contaminated air and soil, where they can be found if plant protection products and organic and mineral fertilizers are used irrationally (Abrahams, 2002; Rajković et al., 2012).

It is difficult to imagine agriculture today and its further progress without the application of various types of pesticides (Knežević et al., 2011). The consequences of their excessive and uncontrolled use are closely related to the withdrawal period and reach the point of creating a potential risk to human health. The withdrawal period is the shortest prescribed time that must elapse from the last application of pesticides to the time of harvest and is indicated on the product (http://pinova.hr/hr_HR/katalog-proizvoda/sredstva-za-zastitu-bilja, access date: 02.09 .2017). Although pesticides are generally expected in foods of plant origin, they simply enter the entire food chain and remain there due to slow decomposition and then accumulate in certain tissues. Aerosols enter the body in the form of vapors, solids, and liquids through the skin, ingestion, or respiratory system (Valić, 2001).

Inhibition of any component of the photosynthetic apparatus will have a negative effect on physiological activities, and therefore most likely on plant growth. Elevated concentrations of toxic metals also affect parameters that show the capacity for photosynthetic gas exchange, such as stomatal conductance. (Li et al., 2013).

Material and methods

Research on the influence of the concentration of a mixture of heavy metals on the dynamics of the accumulation of heavy metals in the wheat stem and plant growth was carried out through experiments in the greenhouse of the Faculty of Agriculture in Zemun, where the heat and humidity were controlled. The vegetation experiment was set up in three repetitions, with a total of 36 pots in which two varieties of wheat, *Pobeda* and *Ljiljana*, were sown. Before sowing, the flasks were filled with 2 kg of Novobalt dry extract, which was

subsequently contaminated with a mixture of chemical compounds of heavy metals in the form of a solution of the following compounds, namely: zinc in the form of zinc-acetate - $Zn(CH_3COO)_2 \times 2H_2O$, lead in the form of lead- acetate $C_4H_6O_4Pb \times 3H_2O$, chromium in the form of chromium trioxide-CrO_3, copper in the form of copper sulfate-$CuSO_4$ and cadmium in the form of cadmium nitrate-$Cd(NO_3)_2 \times 4H_2O$.

The following concentrations of solutions were used:

0 ppm(control), 250 ppm, 500 ppm

Wheat varieties *Pobeda* and *Ljiljana* were chosen because they are mostly grown in the area of southern Banat. 12 seeds were sown in each pot at a depth of 5 cm. Samples from the experiment in pots were analyzed in the budding and leafing stages when four plants were taken from each pot. After sampling the plant, the root was manually separated from the wheat stem. After that, the plant mass - leaves that were previously washed with distilled water and kept for several hours in 0.1 M HCl, in order to remove soil and mineral oxides from the surface. Then the plant mass was ground and dried in an oven at 80°C. 1 g of sample was taken and poured with 20 ml of 60% HNO_3. The mixture was brought to a boil for 2 hours. After cooling, 3 ml of H_2O_2 was added and then boiled for 15 minutes. The procedure was repeated with peroxide. After cooling, 2 ml of $HClO_4$ was added and mild evaporation was carried out until thick white fumes of perchloric acid appeared (Jones and Case, 1990). After cooling, 5 ml of 5M HCl was added, and then the samples were quantitatively transferred into 50 ml volumetric flasks. The flasks were filled to the final volume with distilled water. The solution was filtered through quantitative filter paper. The reading was performed by atomic absorption spectrophotometry (Varian Spectr AA 220FS apparatus), in an acetylene/air flame. The analysis of the obtained data was done with the statistical package STATISTICA 8 for Windows and SPSS Statistics 17.0.

Results and discussion

Stem height

The *Pobeda* variety had a slightly lower average stem height (42.40 cm), while the *Ljiljana* variety had a higher average height (39.67 cm). The average stem height per sample for the *Pobeda* variety varied from 34.00 to 50.60, and for the *Ljiljana* variety from 33.50 to 50.00 (Tables 1 and 2).

Table 1: Stem height, *Pobeda variety*, cm

Variant	Zn, Pb, Cr, Cu, Cd	LSD 5%	LSD 1%
Control	42.60	7.9889	14.6647
250 ppm	34.00	1.8371	3.3723
500 ppm	50.60	1.0625	1.9503
Average	42.40		

Table 2: Stem height, *Ljiljana variety*, cm

Variant	Zn, Pb, Cr, Cu, Cd	LSD 5%	LSD 1%
Control	50.00	1.8371	3.3723
250 ppm	35.50	5.1143	9.3881
500 ppm	33.50	3.3119	6.0795
Average	39.67		

When contaminated with a mixture of metals with a concentration of 250 ppm, the *Ljiljana* variety had a stem height of 35.50 cm, and the *Pobeda* variety was 34.00 cm. When the contamination was carried out with a concentration of 500 ppm, the stem height of the *Ljiljana* variety was 33.50 cm, while that of *Pobeda* was 50.60 cm.

Inhibition of chlorophyll biosynthesis occurs if the plant is exposed to the toxic effects of lead for a long time (Ernst et al., 2000; He et al., 2014). If the lead concentration is higher than 300 ppm, photosynthesis and stomatal conductivity are disturbed (Fu and Wang, 2015).

Leaf surface

Research shows that on average the surface area of the leaves was higher in the *Pobeda* variety (13.40) compared to the *Ljiljana* variety (14.71). The *Pobeda* variety had the smallest leaf area (3.19) in the sample variant where the soil was treated with 250 ppm, while the largest leaf area was in the control sample (31.80) (tables 3 and 4).

Table 3: Leaf surface, *Pobeda variety*, cm

Variant	Zn, Pb, Cr, Cu, Cd	LSD 5%	LSD 1%
Control	31.80	10.1436	18.6201
250 ppm	3.19	0.0183	0.0336
500 ppm	5.23	0.1606	0.2948
Average	13.40		

Table 4: Leaf surface, *Ljiljana variety*, cm

Variant	Zn, Pb, Cr, Cu, Cd	LSD 5%	LSD 1%
Control	13.18	0.6010	1.1032
250 ppm	10.41	4.1425	7.6041
500 ppm	20.54	1.2327	2.2629
Average	14.71		

The research was carried out by setting up vegetation trials in flasks. The uptake of heavy metals in different types of vegetables (lettuce, radish, and carrot) was studied. The soils were contaminated with different concentrations of a mixture of heavy metals. An enormous accumulation of heavy metals occurred in the leaves of examined plants, namely the following metals: iron, zinc, lead, and cadmium. The determined values are above the permitted (MDK) quantities. Somewhat higher contents of iron, zinc, lead, and nickel were found in the tested garden soil samples, but they did not have a negative impact on plant development (Stevanović et al., 2001).

Depending on the chemical properties of the soil, the proportion of adopted heavy metals is different. Thus (Youseff and Chino, 1991) point out that the intensity of uptake of heavy metals is significantly reduced on soils with a pH of 7 and higher. The intake of metals through the leaves is about 10 times higher than through the roots from contaminated soil, with the most efficient uptake of zinc ions through the leaves. The uptake of lead ions from the soil was negligible and the conclusion is that this metal primarily reaches the plants through the leaves from the air.

Conclusion

Vegetation experiments in *v*olumetric flasks were performed with different concentrations of heavy metal mixtures. Increasing the concentration of the metal mixture had a negative effect on the number of leaves and leafy area. Higher concentrations of heavy metals significantly reduced plant growth in both varieties, as well as leaf area in the *Pobeda* variety.

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EQUIPMENT AND USAGE OF TRACTORS IN THE AGRICULTURAL COOPERATIVE "AGROPROM"

Marija Gavrilović[1], Miloš Zelić[2], Biljana Veljković[1], Ranko Koprivica[1], Branislav Dudić[3], Nenad Pavlović[1]

Abstract: Cooperatives as a form of economic activity have become increasingly important in our country in recent years, largely influenced by government incentives in the form of financial aid for the purchase of equipment and machinery and subsidies for the purchase of livestock. In this study, using data on the activity of the agricultural cooperative "Agroprom" from Stara Pazova, the prospects and significance of joining this form of enterprise and functioning according to cooperative principles were analyzed, focusing on the equipment with tractors and their use in primary agricultural production.

Keywords: agricultural cooperatives, agricultural production, mechanization, tractor use.

Introduction

The economic sectors most represented among the 100 largest cooperative enterprises in the world are agriculture and agri-food, trade, and banking. The 100 largest cooperative enterprises in the world employ nearly 3 million people (2,987,372 employees), with just over 2.5 million in Europe.) (www.enterprises.coop).

Most agricultural producers in Serbia could not compete in terms of quantity and quality of their products, but with the help of joining cooperatives they found their place in the market. By joining cooperatives, they also received

[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia (marija.gavrilovic@kg.ac.rs)
[2]University of Belgrade, Faculty of Agriculture, Nemanjina 6, Zemun, Serbia (miloszelic7@gmail.com)
[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia (biljavz@kg.ac.rs)
[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia (ranko@kg.ac.rs)
[3]Comenius University in Bratislava, Faculty of Management, P.O. Box 95, Odbijarov 10, Bratislava (branislavdusic@fm.uniba.sk)
[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia (nenadpavlovic@kg.ac.rs)

a number of benefits such as cheaper procurement of raw materials and machinery, as well as help in bookkeeping (Koprivica et al., 2013; Gulan, 2019).

The agricultural cooperative "Agroprom" from Stara Pazova is a legal entity established according to cooperative principles. It was established in 2000. The cooperative is engaged in the production, purchase and sale of agricultural products such as corn, wheat, soybean, sunflower, rapeseed and sugar beet. Currently, the cooperative occupies a very important place in the field of agriculture in the territory of the Srem district and, together with some agricultural companies and private individuals, plays a dominant role in the purchase of agricultural products.

Materials and methods

Data collection on the agricultural cooperative was conducted using the interview method. Questions were asked and answers with concrete data were obtained from the professional service of the cooperative. Analytical synthetic methods were used in analyzing the business activities of the agricultural cooperative, and the obtained data were used to calculate the production dynamics and business performance.

The data sources were national and international literature, laws and other legislation, annual reports of the organization, various internal documents of the cooperative, as well as data obtained from the official websites of relevant organizations. The main data source used was the documentation of the specialized service of the cooperative "Agroprom" Stara Pazova.

Results and discussion

The best picture of the situation of cooperatives in Serbia comes from the analysis of all financial reports received, with over 1,500 cooperatives having submitted financial reports to the Agency for Business Registers at the end of 2015
(Zakić and Nikolić, 2018; Veljković et al., 2020). From July 2017, when the action "500 Cooperatives in 500 Villages" was launched, until the end of 2020, about 830 new cooperatives were established in Serbia. Under the "500 Cooperatives in 500 Villages" campaign launched in mid-2017, by the beginning of 2021, 1.7 billion dinars will be invested in the reconstruction of cooperatives in Serbia. The money was used to improve the lives of 6,120 rural families or about 30,000

inhabitants in the villages. Most of the cooperatives are engaged in agricultural production – 62.51% (www.zssrbije.org).

The cooperative "Agroprom" Stara Pazova has been operating without interruption since its establishment on July 17, 2000. Most of the subcontractors with whom the cooperative cooperates are located in the territory of the municipality of Stara Pazova, but it also has subcontractors in other municipalities of the Srem region. The production is based exclusively on the production of field crops. Besides the production of agricultural products, the cooperative also deals with the purchase, storage and sale of agricultural products. The cooperative operates according to cooperative principles.

From the very beginning, the cooperative has been one of the largest sugar beet producers in Vojvodina. Today, the cooperative has a total of 340 ha of its own and leased agricultural land, while it produces with subcontractors on about 3,800 ha. The sowing structure in 2019 is shown in Table 1.

Table 1. Sowing structure in 2019.

Crop	Area (ha)	Average yield (t ha^{-1})	Total production (t)
Wheat	135	11.99	1618
Corn	1835	12.54	23007
Soybean	132	8.68	1146
Sunflower	282	7.32	2063
Rapeseed	208	1.15	240
Sugar beet	302	84.00	25368
Total	2592		

Production on owned and leased agricultural land is unthinkable without appropriate tools and mechanization of agriculture. It is important to mention that the cooperative owns a silo with a capacity of 16,000 t, and it is planned to build another silo with a capacity of 8,000 t, as the current facility is not sufficient for storing the quantities produced. In addition, the cooperative owns a farm with a total area of 2 ha, where, in addition to the silos, machinery and part of the raw materials needed for production (seeds, fertilizers, pesticides) are stored. The cooperative is fully equipped with the machinery necessary for agricultural production. The list of machines owned by the cooperative and their number are shown in Table 2.

This table gives a complete overview of the mechanization means that the cooperative has at its disposal and that are necessary for the execution of all agrotechnical works in agricultural production, from basic and complementary tillage to sowing, maintenance, protection, harvesting, transport and storage.

Table 2. Specification of mechanization

Means of mechanization	Number
Tractors (all categories)	35
Combine harvesters (grain)	14
Combine harvester (tail)	5
Beet cleaners	2
Trucks	6
Set-top boxes	8
Planters (maize, sugar beet, sunflower)	8
Universal seed drill Horse(wheat, soybean, rapeseed)	1
Multi-furrow plows	12
Sprayer 3000 l - trailed	8
Self-propelled sprayer - high spacing	1
12-furrow inter-row cultivator	5
Heavy plows with packer rollers	7
Subversive	4
Heavy harrows	4
Mineral fertilizer spreader	4
Watering can	1
Corn adapter	10
Tarup	2

The degree of labor productivity, the efficiency of production, and the profitability of the farm also depends on the equipment of the farm with means of production (Zimmer, 2019). Production costs, of course, include the cost of maintaining machinery, repairs and spare parts (Koprivica et al., 2020). It is particularly important to mention the number of tractors, whose types and percentage representation by manufacturer name are presented in Table 3.

In order for agricultural production to run smoothly and for all agro-technical work to be carried out on time and to a high standard, tractors of varying power are needed, which in this case was fulfilled. Among agricultural cooperatives in Vojvodina, tractors with medium power are the most common, accounting for 55%. 25% are low power tractors and 20% are high power machines (www.zssrbije.org)

Table 3. Numerical condition of tractors

No.	Type of tractor	Number	Category	Share (%)
1.	IMT-542	1	LP*	2.86
2.	Lamborghini Premium 1060	2	MP**	5.71
3.	Lamborghini Premium 950	2	MP	5.71
4.	Belarus MTZ-892	1	MP	2.86
5.	Belarus MTZ-1025	8	MP	22.86
6.	John Deere 6620	6	MP	17.14
7.	John Deere 6630	3	MP	8.57
8.	John Deere 6530	2	MP	5.71
9.	Telehandler JCB 531-70	1	MP	2.86
10.	Landini Ghibli 100	1	MP	2.86
11.	Case IH Maxxum 115 A	2	MP	5.71
12.	John Deere 8330	3	HP***	8.57
13.	John Deere 8420	1	HP	2.86
14.	Fendt 930	2	HP	5.71
Total		35		100

*LP-low power tractor; **MP-medium power tractor; ***HP-high power tractor.

With the help of adequate and modern mechanization, tractors and equipment, the cooperative realizes a significant source of income by providing services to other agricultural producers. The income from services in the period from 2017 to 2019 amounted to RSD 90,846,463, RSD 86,520,241 and RSD 50,558,104, respectively, indicating that other producers should also equip themselves with their own machinery and reorient towards greater investment in production in relation to service activities.

Conclusion

Cooperatives have great potential for sustainable economic and social development in Serbia. Taking into account international experience, it is clear that cooperatives have various economic, economic and social advantages compared to other forms of enterprises and organizations. Cooperatives have great potential for creating safer and more sustainable enterprises, they function according to a democratic management model, where cooperative members participate equally in the management and have equal rights.

Agricultural cooperatives are key factors in the process of renewing agricultural mechanization, but they are underutilized. A large number of cooperatives in Serbia are relatively well equipped with machinery, but due to their long-time use, the need for renewal is always obvious. On the other hand,

there are cooperatives that still need to be mechanized, so every third cooperative needs a new tractor or new equipment. In cooperative mechanization, it is crucial that farmers can more easily access high-quality machinery through the cooperatives, as it is difficult to obtain it independently. With the help of the cooperative, cooperative members can acquire higher quality and more diverse farm machinery that is sufficient for all their needs. In most cases it is not rational for the farmer to buy all the necessary machinery himself, but it is more economically justified and logical to do so with common means. This is exactly the experience in the agricultural cooperative Agroprom, where there is no lack of good business results.

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INVESTIGATION OF ZINC CONTENT IN AGRICULTURAL LAND IN THE AREA OF THE CITY OF SOMBOR

Danijela Žunić[1], Vladimir Sabadoš[2]

Abstract: Zinc belongs to the group of heavy metals, it is an integral part of a large number of fragments. The toxicity of heavy metals is reflected in morphological and anatomical changes in living tissues, while on the other hand, efficient absorption of essential metals is necessary for the normal growth and development of plants and animals. Agricultural Extension Service analyzed the agricultural land for zinc content in the soil, and it was determined that it ranged from 26.76 mg/kg to 228.20 mg/kg. The most common zinc content at a depth of 0 to 30 cm is in the range of 60 to 80 mg/kg, in 31.41% of the tested samples of agricultural land in the area of the city of Sombor, while for the zinc content below 30 mg/kg, only 0.64 % of the country.

Keywords: zinc, heavy metals, land, concentration, essential metals

Introduction

Zinc is widely distributed in nature, but in small concentrations. Zinc is in the II B group of chemical elements together with cadmium and mercury. Zinc belongs to the group of heavy metals whose specific density is greater than 5 g/cm3 (Bell at.al. 2008). The concentration of zinc in the lithosphere, the earth's crust, ranges from 20 to 100, with an average of about 80 mg/kg. In the surface layer of the soil, the concentration of zinc most often ranges from 10 to 300, and on average around 60 mg/kg (Kastori et.al., 2020). Kabata - Pendias (2011) state that the concentration of zinc in the surface layers of different types of soil in the world ranges up to 125 mg/kg.

The concentration of total zinc in certain soil types varies widely. It is the lowest in light sandy soils, on average around 30 mg/kg. In acidic sandy soils with intensive leaching, the concentration of zinc can be even lower, of 30 mg/kg. In soils of the chernozem type, it usually ranges from 120 to 150 mg/kg, and in forest soils from 70 to 120 mg/kg (Kastori et.al., 2020). The influence of individual elements on the life process and thus the growth and development of plants also depends on their concentration.

[1]Agriculture Extension Service „Sombor", Staparski put 35, Sombor, Serbia (agroso@mts.rs)

A high concentration of zinc, like other heavy metals, has a toxic effect on plants. Approximately 6 million tons of zinc circulates annually in nature, carried by wind, running water and precipitation. The anthropogenic emission of zinc in the atmosphere as a result of human activity amounts to 62,000 tons per year. It is considered that only about 10% of zinc circulating in nature and anthropogenically formed is accessible to living organisms. An excess of this element rarely occurs in plants in nature. This phenomenon can most often be observed in the vicinity of zinc mines and smelters, where its content in the soil can reach as much as 3-4% (Kastori et.al., 2020). Accumulation of zinc in the soil can also occur during regular fertilizing with a large amount of manure, especially pig dung or compost from urban waste, during the wear of motor vehicle tires, corrosion processes, when burning coal and urban and industrial waste material (Sabadoš et.al. 2019).

Agricultural land in he town of Sombor

The total area of available land in the territory of the city of Sombor, according to the agricultural census from 2012, is 122,948 hectares. Of this, 100,381 hectares are agricultural land, while 22,567 hectares belong to "other land" (forest and other lands). This land is used, according to data from the census, by 8,372 agricultural holdings (Sabadoš et.al. 2019). The most common type of agricultural land in the area of the city of Sombor is chernozem with all its types and covers 46.9% of the total area of the city of Sombor. In second place is the meadow black, which is represented at 35.2%. Rit black land covers 6.0%, alluvial land covers 5.9%, and fens and marshy lands comprise 5.0% of the total area of the city of Sombor. Sandy soils are represented on 1.0% of the surface (Sabadoš et.al. 2019).

Materials and methods

In this survey of agricultural land in the area of the city of Sombor, the content of the basic parameters of soil fertility was examined at the depths of 0 to 30 and 30 to 60 cm, while the zinc content was examined at the depth of 0 to 30 cm. Laboratory analyzes of the basic parameters of soil fertility were performed in the laboratory of the Agricultural Expert Service Sombor in Sombor, while analyzes of the zinc content were performed in the laboratory of the Institute for Crop and Vegetable Production in Novi Sad.

Land sampling was carried out at fixed points with the determination of GPS coordinates for each point individually, which contains the cartographic basis for the area of the city of Sombor where a triangulation network with a list of GPS coordinates is presented permanent points on agricultural land in the area of the city of Sombor.

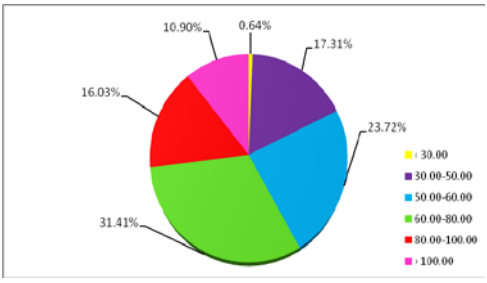
The method used to determine the zinc content in soil is Optical Emission Spectrometry with Inductively Coupled Plasma ICP-OES. In the ICP-OES method (Inductively Coupled Plasma Optical Emission Spectrometry), the sample is introduced into the plasma source where it vaporizes and decomposes into free atoms and ions, whereby additional energy is consumed in order to excite the free atoms and ions into high energy states.

Results and discussion

According to the analyzes of agricultural land in the area of the city of Sombor for the zinc content in the soil, it was determined that it ranged from 26.76 mg/kg to 228.20 mg/kg. The mean values of this soil fertility parameter in the 0-30 cm layer are 71.34 mg/kg, and the maximum determined value is 228.20 mg/kg in the sample from point number 70 in the area of the Nenadić farming settlement. This zinc content is far from the maximum permissible amount of zinc in the soil.

The most common content of zinc at a depth of 0-30 cm is in the range of 60-80 mg/kg, and that is in 31.41% of the examined samples of agricultural land in the area of the city of Sombor, while with a zinc content below 30 mg/kg, only 0 .64% of the land.

Graph number 1: Percentage representation of tested samples of agricultural land in relation to zinc content (mg/kg) in the area of the city of Sombor



Regarding the zinc content in the soil in the layer of 0-30 cm, and based on the type of soil, the highest average zinc content is found in alluvial soils, 85.56 mg/kg, while the lowest average zinc content is 65.33 mg/kg of land (graph number 1).

Graph number 2: Average zinc content (mg/kg) by type of agricultural land in the area of the city of Sombor

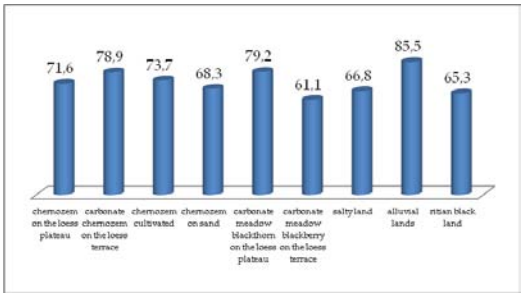
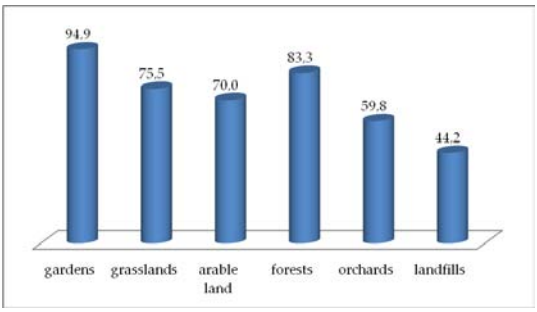


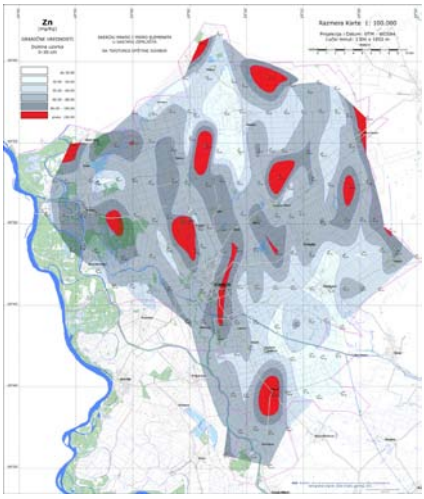
Chart number 3: Average zinc content (mg/kg) in the 0-30 cm layer of agricultural land in the area of the city of Sombor



Observing the content of zinc in the soil in samples of agricultural land by ownership, it can be concluded that the highest content of zinc in the land is in state ownership (chart number 4).

The distribution of zinc content in soils in the 0-30 cm layer in the area of the city of Sombor is given on map number 1.

Map No. 1: Values of zinc content (mg/kg) at a depth of 0-30 cm in the area of the city of Sombor



Conclusion

Based on the analysis and research of agricultural land in the area of the city of Sombor for zinc content, it can be concluded that there is a wide spectrum of zinc content in the soil. The range was from 26.76 mg/kg to 228.20 mg/kg. The cause of the high level of zinc in the soil is unknown, but it is assumed that some of the samples of this amount are uncontrolled discharge into the groundwater, excessive fertilization of the plot with fertilizer, as well as the possibility of a large amount of exhaust gases with zinc circulating in nature, and with it soil contamination and the adoption of plant flanks. The results show that zinc in the soil is within the normal range of 60 mg/kg to 80 mg/kg in the area of the city of Sombor, which shows that in our area we still have sufficient zinc reserves compared to the rest of the world. This data also tells us that farmers still take care of the method of fertilization, crop rotation, etc. However, zinc is one of the few elements whose toxicity is to a lesser extent precisely because of its lack in the soil. It has been calculated that about one-third of the world's agricultural land, as well as crops and human population, is insufficient due to low soil content. Regulations for determining the limit values of critical metal concentrations, the so-called "maximum permissible concentrations", differ from country to country, in order to meet the different environmental conditions of each region and country in the world.

Small amounts of zinc were found at 0.64%, which is an extremely small percentage, and in those places biofortification is used in order to improve their nutritional quality. Agronomic biofortification means fertilizing plants with microelements in order to increase their concentration in the edible parts of the plant. Biofortification with zinc is one of the ways to increase the presence of this essential element in people's diet, thus avoiding the possible consequences of its deficiency. Recently, various biostimulators or biological fertilizers, which can be used in biofortification, have become increasingly popular.

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ANALYSIS OF THE MEANING AND IMPACT OF SUBSIDIES ON THE DEVELOPMENT OF TOBACCO PRODUCTION IN MACEDONIA

Silvana Pashovska[1], Katerina Kareska[1]

Abstract: With the payment of subsidies in agriculture, the Republic of North Macedonia remains consistent in supporting the development and advancement of the agricultural sector and specifically tobacco production as one of the main branches of the Macedonian economy. Of course, it is necessary to comply with the common agricultural policy of the European Union, which implies a change in domestic legislation. More specifically, it is a matter of passing three key laws for harmonizing national policies with EU agricultural policies, that is, the Law on Direct Payments, the Law on Rural Development and the Law on Regulation of Markets.

Keywords: agricultural policy, incentives, inflation, tobacco production, global flows

Introduction

Macedonia is a country in which quality types of oriental tobacco are traditionally grown, in addition to the famous regions in Turkey, Izmir and the Sea of Marmara area, where there are ecological conditions for the production of high quality oriental tobaccos. As a small continental country in the Balkans, Macedonia represents a "golden triangle" in the production of oriental tobaccos and has been a relatively stable traditional producer for many years in relation to the countries that surround it, which are producers of this type of tobacco.

Tobacco is a fully exportable product, the purchase of which depends directly on the requirements of multinational companies that have registered companies in Macedonia, so the volume of contracted production and the height of the market price derives from the raw material needs of the global cigarette industry. Modern ways of doing business gradually impose the need to consolidate the commercialized family enterprises where tobacco is mainly

[1]University St. Kliment Ohridski – Bitola, Scientific tobacco institute – Prilep, Republic of North Macedonia, e-mail: silvana.pasoska@uklo.edu.mk, e-mail: katerina.kareska@uklo.edu.mk

produced. It is considered that 10% of the population in Macedonia sees their financial survival in the production of tobacco.

Macedonia is considered a producer of high-quality aromatic oriental tobacco both regionally and globally, on world stock exchanges and markets. Tobacco production accounts for 5% of total exports and almost 30% of exports in the agricultural sector. The demand for Macedonian oriental tobacco (it is also found in the neighboring countries Bulgaria, Greece and Turkey, but not with the same quality as the Macedonian one) is still high. Tobacco production as an agricultural branch acquires strategic importance for the state economy, as a significant item of the earned income directly flows into the state budget.

This points to the fact that the future development of tobacco production and the entire complex of activities related to it must be based on the preferred standards of the European Union and the currents of modern world achievements in that area. The preferred standards of future development point to greater attention to the production itself, both to its quantitative limitation and to its qualitative worthiness, environmental protection, social security, economic efficiency, a healthy way of growing and developing plants, as well as respect to the guidelines from the World Health Organization.

In the European Union, if farmers do not meet the set standards, the amounts for direct payments (aid or subsidy) are reduced or completely canceled in the year in which those standards are not met.

The future of tobacco production will mostly depend on a good agrarian and export policy, but also on a good subsidy policy by the state, all in order to encourage and direct the young able-bodied population to tobacco production, in which they will see not only an opportunity for livelihood, but also space for development, expansion and of course making a profit. In that direction, motivation and leadership are a necessary link in a series of other related activities for realizing the entrepreneurial spirit among young people who are the future of our country. If all the measures continuously offered by the European commissions for the protection and promotion of agriculture, including the IPARD funds, are used, the development course of tobacco production in our country will be greatly advanced.

This means that among the population that is oriented to agricultural production and above all to tobacco production, it is of primary importance to create and build a perception of security and future in this sector, for which a developed management of all activities and appropriate motivation is necessary. Positive stimulation and motivation will ensure greater efficiency

and effectiveness in work, reduction of production costs, increase in productivity and improvement of work results.

Material and methods

The research in this paper has a scientific and analytical approach with mandatory use of statistical data from relevant sources such as: World Bank, State Statistics Office of the Republic of North Macedonia, Ministry of Agriculture, Forestry and Water Management of the Republic of North Macedonia, Chamber of Commerce, data from Eurostat database, data from the Food and Agriculture Organization of the United Nations - FAO (Food and Agriculture Organization of the United Nations), data from the World Health Organization, as well as own analyzes and empirical research through the use of mathematical-statistical and comparative methods, as well as tabular and graphical presentation of the obtained results.

Results and discussion

The Republic of North Macedonia continuously supports the subsidization of tobacco production and tobacco is a unique crop whose production and purchase is regulated by the precise Law on tobacco, tobacco products and related products. According to the analysis of the Ministry of Agriculture, Forestry and Water Management of the Republic of Macedonia, there is no payment of subsidies for tobacco in Turkey, and in Bulgaria and Greece subsidies for the production of oriental tobacco were last paid in 2009. The existing conventions and initiatives by the world's health institutions are not a threat to reduce the areas with tobacco, and according to experts, the development of this production is going in the right direction.

With regular payment of subsidies to farmers, the state remains consistent in supporting agriculture and specifically tobacco production as one of the main branches of the Macedonian economy. Of course, it is necessary to comply with the common agricultural policy of the European Union, which implies a change in domestic legislation. More specifically, it is a matter of passing three key laws for harmonizing national policies with EU agricultural policies, that is, the Law on Direct Payments, the Law on Rural Development and the Law on Regulation of Markets.

In order to get a clearer picture of the effect of subsidies on agriculture, the Ministry of Agriculture, Forestry and Water Management of the Republic of

Macedonia plans to conduct a detailed analysis, including all the expert and professional capacities that the state has at its disposal, which will data will be obtained for the creation of further agricultural policies and for the first time it will be known exactly which crops are strategic and whether there will be a need for additional financing and stimulation.

Regarding the economic effect of subsidies, the question is often asked: Do subsidies further feed the inflation that occurs as a reflection of global flows? It should be emphasized here that inflationary effects may have a limited direct impact, why subsidies cannot be the cause of product price fluctuations because fluctuations mostly depend on the effects of the overall fiscal and monetary policy. Subsidies as a stimulus measure in agriculture, and thus in tobacco production, do not have a measurable effect on local development, but through investments in mechanization, processing facilities, infrastructure, etc., they can be a stimulus and an incentive for development. Greater awareness, training and education of the agricultural population and companies from this area would lead to their greater utilization and rational use. In Macedonia, in the field of tobacco production, the following amounts have been allocated in the form of subsidies (Table 1).

Table 1. Share of direct payments for tobacco in total direct payments for agriculture, 2015 - 2021

Year	Total funds for financial support in agriculture in denars	Total funds for produced and sold raw tobacco from harvests 2015-2021 in denars	Participation of tobacco support in the total support of agriculture in %
2021	3.723.103.000	1.276.178.716	34,3
2020	6.668.000.000	1.724.500.000	25,9
2019	6.138.767.000	1.699.679.706	27,7
2018	6.210.500.000	1.696.688.814	27,3
2017	6.117.857.000	1.505.429.595	24,6
2016	6.320.000.000	1.130.548.658	17,9
2015	6.260.000.000	1.489.031.604	23,8

Source: Ministry of Agriculture, Forestry and Water Management of the Republic of North Macedonia

Table 2. Share of direct payments in total value per kilogram

Year	Average purchase price kg/denars	Average subsidy per kilogram	Total value	Price in euros (kg/purchased tobacco)	% of the subsidy in the cost price
2010	136.6	60	196.6	3.20	31%
2011	164.8	60	224.8	3.66	27%
2012	180.2	60	240.2	3.91	25%
2013	152.6	60	212.6	3.46	28%
2014	117.2	60	177.2	2.88	34%
2015	184.5	60	244.5	3.98	25%
2016	196.8	60	256.8	4.18	23%
2017	217.6	70	287.6	4.68	24%
2018	214.2	70	284.2	4.62	25%
2019	219.9	70	289.9	4.71	24%
2020	158.8	70	228.8	3.72	31%
2021	200.4	70	270.4	4.40	26%

Source: Ministry of Agriculture, Forestry and Water Management of the Republic of North Macedonia

In the calculation, it is assumed that after 2017, an average of 70 denars subsidies were paid per kilogram of purchased tobacco, because the second class participates with more than 60% in the purchase, and the first and third class with slightly less than 20%. From the calculations, it can be seen that the percentage of participation in the total price received by the producers is 23% to 31%, which is a significantly high percentage (Table 2., Chart 1.)

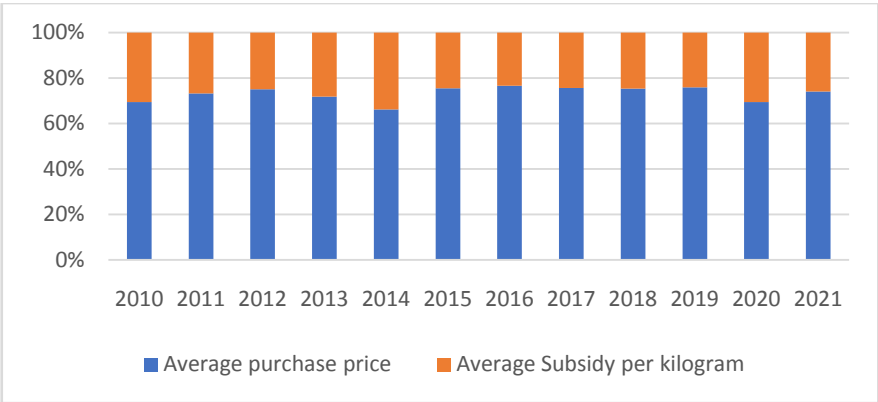

Chart 1. Graphic representation of the participation of subsidies in the total price of a kilogram of delivered purchased tobacco by years

In order to give an assessment of the impact of subsidies on agriculture, and especially on tobacco production, it is necessary to take into account the fact that in Macedonia since its independence, only one census of agriculture has been carried out, in 2007, while in 2022, the so-called A trial census of agriculture that will be the basis for the main census that will be carried out during 2023. What is still a big drawback in the agricultural subsidies in our country is that a large part of the subsidies are used as a social measure, although they should contribute to increasing competitiveness, to the creation of large carriers of the production process that will then contribute to increase in exports. In this direction, special attention should be paid to how the subsidies cause permanent positive consequences, and not temporary and apparent effects.

If we continue with large expenditures in agriculture in the form of subsidies, the thesis is asserted that in conditions of increased subsidies as a global trend of rising prices, it causes the release of a mass of money for consumption, instead of increasing consumption for investments in infrastructure that does not would cause inflationary movements. If this is the case, the issue of limiting their use or restructuring the subsidy plan also arises.

Every year the Government of the Republic of North Macedonia pays out significant funds from the central budget in the name of subsidizing agricultural production (Chart 2.) Most of these funds are in the form of direct payments to farmers and are seen as a key tool for maintaining Macedonian agricultural production. This type of direct financial assistance for many farmers means supplementing their incomes, which on the other hand directly affects the profitability of the agricultural activity. For more than a decade, subsidies have been one of the key agricultural production support measures of all governments, regardless of their political background. Direct payments in agriculture are divided into direct payments for crop production, direct payments for livestock production and direct payments for organic production. In addition to these forms of direct payments in agriculture, in certain years there are measures for additional support for the development of agriculture, such as financial support for insurance, for the protection of agricultural land, for providing technical support in agriculture, as well as assistance for certain categories of holders of agricultural holdings. (for example, a young farmer or for increasing arable land, etc.). For the period 2008-2019, 1.049 billion euros were paid in agriculture for agricultural subsidies, which is 87 million euros on average per year.

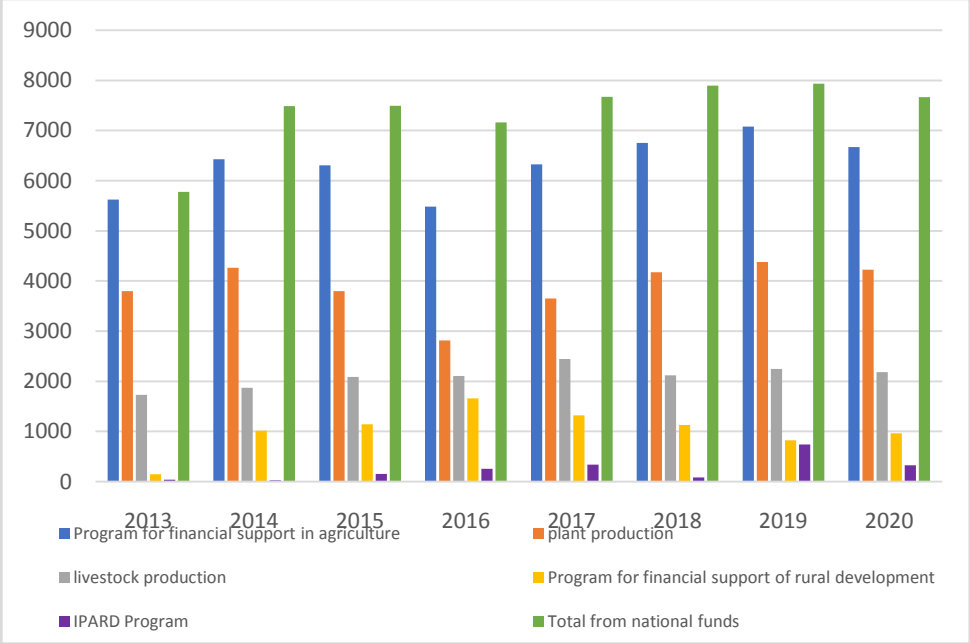

Chart 2. Programs for financial support in agriculture and rural development - subsidies for crop and livestock production and the IPARD program in the period 2013 - 2020

Source>National strategy for agriculture and rural development for the period. 2021-2027

The Tables shows that Macedonia constantly allocates funds for financial support of agriculture and that most of the disbursed funds are paid in the form of direct payments to farmers, which for tobacco producers are based on delivered kilo by class. The fact is that Macedonia, as a candidate country for membership in the European Union, will have to comply with the Union's Common Agricultural Policy (CAP), which includes a gradual transition from direct support per paid kilogram to direct support based on arable hectares. However, in its recent National Tobacco Strategy, the government confirmed that production-related targeted direct payments will continue to be used for some sub-sectors, including tobacco production.

According to today's state of the economy, instead of developing, increasing production, purchasing and modernizing equipment or expanding the activity, the largest percentage of received subsidies are used by tobacco producers to supplement their own budget. Actually, instead of a development component, the subsidies do not give the expected result and represent a component for

"maintaining life". It is understood that in order to have an effect from them, they should first go into the right hands, i.e. with the primary producers, then parallel to the primary tobacco production, they should to develop the processing industry. In our country, the processing facilities for processing tobacco and tobacco products are predominantly privately owned, and tobacco, which is almost entirely an export product, depends directly on the requirements of multinational companies that have registered companies in Macedonia. Hence, the scope of the agreed production and the height of the market price results from the raw material needs of the world cigarette industry. In recent years the demand for tobacco is on the downward trend, but not only for tobacco but also for the world market the global demand for cigarettes is decreasing which leads to a decrease in the demand for unfermented tobacco, and that brought it also leads to a reduction in tobacco prices. Due to its quality and exceptional properties, especially due to the fact that oriental tobaccos cannot be easily replaced in cigarettes, Macedonian tobacco is still in demand in the markets of the world.

Currently, subsidies for tobacco production largely have an economic component and most of the time, instead of development, they serve to supplement the domestic budget and a significant number of families rely on tobacco production as the main source of income. Most tobacco producers are directly dependent on government subsidies, but in the medium and long term this type of policy is unlikely to be economically sustainable.

Despite the high subsidies, the average monthly income of tobacco producers is lower than the average monthly net salary in Macedonia and far below the value of the minimum consumption basket of households.

The danger hidden in the subsidies thus generated is that farmers may decide to grow crops only because of the high subsidies. As a result, more tobacco can be produced than the market can absorb.

Despite the amount of subsidies, in Macedonia, the number of concluded contracts, the number of tobacco producers and the number of hectares under tobacco are decreasing (Chart 3.) The reason for this probably lies in the fact that tobacco, as a distinctly labor-intensive crop, is not interesting for the young population, as well as the fact that the average salary in agriculture is lower than the average salary in the country, by almost a quarter

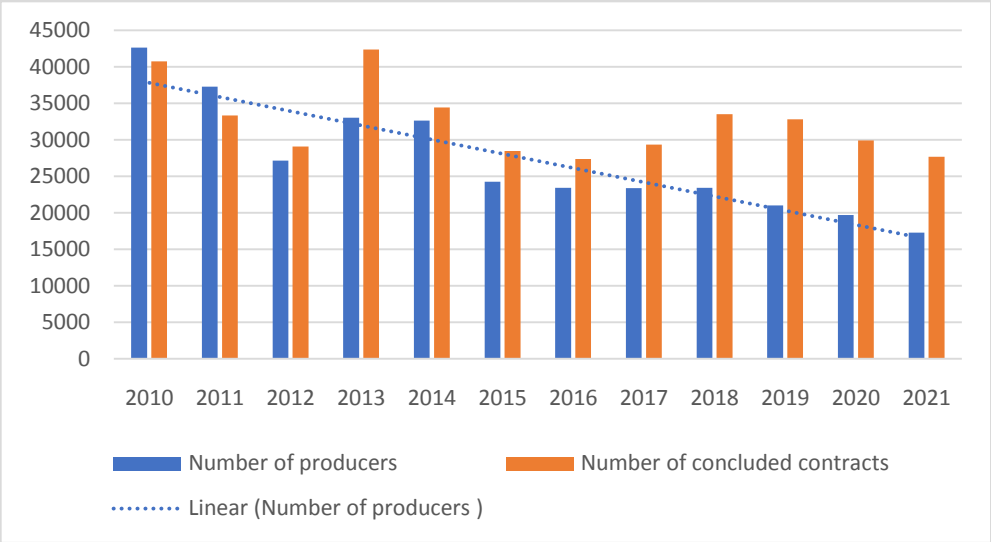

Chart 3. Number of signed contracts and number of tobacco producers

Source: Ministry of Agriculture, Forestry and Water Management of the Republic of North Macedonia

This can be followed by the fact that perhaps some of the tobacco producers are thinking about diversifying their production towards other crops or choosing an alternative production. Thus, from several analyzes and interviews of tobacco producers, the result is that 30% of them are ready to redirect their production in accordance with the requirements that may arise during the EU accession process. This percentage is significant and can be increased if the state works to educate and inform tobacco producers.

Conclusion

Based on the previously presented situations and analyzes of the meaning and impact of subsidies on the development and sustainability of tobacco production, we can state that in order to increase the effect of subsidies, it is necessary to move them within the following framework:

• Macedonia, as a candidate country for membership in the European Union, must respect the EU's Common Agricultural Policy (CAP), which includes a gradual transition to direct support based on planted hectares and not on the amount of agricultural crops. It will also be necessary to think about diversification of production, and the new National Tobacco Strategy 2021-2027 provides several approaches, i.e. possible exit solutions for replacing tobacco

with other crops (such as peppers, hazelnuts, stevia, sesame, etc.) . Diversification, primarily due to traditionalism, would go slowly and for that, a lot of education, time and effort invested not only by tobacco producers, but also by all relevant institutions is needed.

- Subsidies must contribute to increased productivity and efficiency which indicates the fact that the state should carry out a thorough mapping of tobacco producers in order to distinguish those who are for professional agriculture from farmers who only have short-term goals to grow a certain crop such as tobacco only when there are subsidies for that crop. It is also necessary to work on the inclusion of the young population, who could more easily and effectively adapt to the use of new technology and innovations with tobacco production, which would increase the efficiency and effectiveness of tobacco production entities and lead to greater productivity.

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CROP WEEDS DETECTION USING NEURAL NETWORK MODELS

Dušan Marković[1], Uroš Pešović[2], Dalibor Tomić[1], Vladeta Stevović[1]

Abstract: Weeds are one of the most important factors affecting agricultural production. Environmental pollution caused by the application of herbicides over the entire agricultural land surface is becoming more and more obvious. Accurately distinguishing crops from weeds by machines and achieving precise treatment of only weed species is one possibility to reduce the use of herbicides. However, precise treatment depends on the precise identification and location of weeds and cultivated plants. The aim of the work was to describe and point out the importance of deep learning models for the detection and classification of weeds, in order to enhance their application in real conditions.

Keywords: agriculture, image processing, artificial neural network, weed detection, weed control

Introduction

Nowadays, there is an initiative to automate, speed up and synchronize many processes with the help of new smart technologies. As in other areas, it happens in agriculture as well. In this way, the reduction of labor consumption is achieved while at the same time increasing the productivity of agricultural production (O'Donoghue et al., 2011, Wu et al., 2021). The aforementioned progress is due to the introduction of new technologies in agricultural production (GPS, robots, etc.).

Trends in agriculture today are directed towards food production in an ecologically acceptable way while simultaneously preserving the environment. On the one hand, the demands are to increase agricultural production with greater competitiveness, and on the other hand, the production of health-safe food with less use of chemicals is required. All the stated goals are difficult to fulfill, but it is possible, especially if the concept of precision agriculture that optimizes the use of resources is included in the production process.

The precise weed control systems developed so far cannot yet completely replace conventional systems, but they are striving for them and achieving their

[1]University of Kragujevac, Faculty of Agronomy Čačak, Cara Dušana 34, 32102 Čačak, Serbia (dusan.markovic@kg.ac.rs)

[2]University of Kragujevac, Faculty of Technical Sciences, Svetog Save 65, 32102 Čačak, Serbia

partial replacement. The basis for further progress is the cooperation of agronomists, information technologies, and mechanical engineers (science, technology, engineering, and mathematics).

One of the most important steps in the adequate application of precise weed control systems is the precise detection or classification of weeds in the field. This implies the precise determination of weed and cultivated species and the detection of the place where the plants are located. On the basis of these data, further decisions are made about the way of working of precise constructions of machines for their suppression (Wang et al., 2019).

For the purposes of weed detection, various deep learning models are very useful. They can be divided into two categories: conventional machine learning-based classification and deep learning-based classification. Machine learning refers to a group of computerized modeling approaches that can learn patterns from the data so as to make decisions automatically without programming explicit rules (Singh et al., 2016; Wang et al., 2019).

The aim of the work was to describe and point out the importance of deep learning models for the detection and classification of weeds, in order to enhance their application in real conditions.

Deep learning model

Detection of crops from captured images is performed by a pre-trained deep learning model. Deep learning is a part of artificial intelligence that focuses on solving problems using neural networks which are trained using large amounts of data. A neural network consists of artificial neurons organized in layers, where there is an input layer, one or more hidden layers, and an output layer. Each layer contains artificial neurons, known as nodes, which are connected with the nodes in the next layer. Each node has two parameters weights and threshold, where weight is defined during the training phase. The weights parameter for each node is updated for every known input and output data during training and represents a learning mechanism. During the training, the accuracy of the neural network improves until it reaches sufficient accuracy to predict the output or to cluster and classify new input data.

Our proposed model was defined as a neural network for classification images implemented with the Keras framework. Keras has a high level of abstraction, which is relatively easy to use due it is written in Python programming language. Keras build on top of the TensorFlow framework and could easily use all its capabilities and cover every aspect of the learning

workflow. TensorFlow is an open-source platform for machine learning that has an entire ecosystem of tools and libraries with adequate community support. All these resources allow the development of applications based on machine learning by data scientists and data engineers (Keras, 2023).

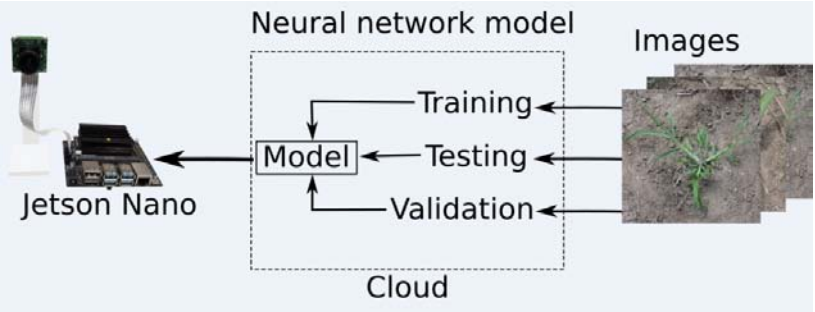

Figure 1. Prepering Neural network model for Jetson Nano

The weeds images are split into the images for the training set and two smaller set for testing and validation, which were loaded into the model using Keras framework. Usually some preparation of data is necessary which would be input to the first layer in implemented neural network. Then the parameters of the neural network are defined and training process is conducted. The entire model finding process should take place in the cloud using an infrastructure with sufficiently high performance. After training phase, model was tested against unused data to check accuracy of obtain model. If the result of testing phase is satisfactory then train model could be used to classify weeds or recognize weeds or benefit crops. Then entire model is saved in appropriate format than it could be transfer to field devices used to detect weeds (Figure 1).

Deep learning applications are based on matrix computations which require computers with high processing power. Field devices, on the other hand, have a set of spatial and power consumption constraints, so they need to be energy efficient to execute such high-performance deep learning applications. Raspberry PI single-board computer was first used in such applications, but its real-time performance in image processing applications was very limited to less than one frame of video per second. The main reason for the poor performance of Raspebby PI's dual-core ARM processor is that it was not designed for fast matrix operations. Raspberry Pi performance was slightly improved in such applications using Intel Neural Compute USB Stick, which was used to offload the processor from matrix intense operations. Graphical processing units have dozens of cores, which makes them more suitable for matrix operation when

compared to the central processor. NVIDIA, a leading manufacturer in graphics processing units, launched the Jetson Nano computer board with a built-in 128-core Nvidia GPU for accelerating deep learning applications. Jetson Nano has quad-core ARM central processor which with assistance of integrated 128-core NVIDIA Maxwell GPU could reach performance level of 472 GFLOPS, or 472 billion floating point operations per second. Such performance levels are dozen times better when compared to Raspbeery PI, which makes Jetson Nano suitable for real-time image processing applications as shown by Assunção et al. (2022). Jetson Nano has small dimensions of 100x79 mm and power consumption of 5-10W so it can be powered by rechargeable battery. It runs Linux operating system which is preloaded onto the SD memory card.

Application of deep learning models in agriculture

Weeds are one of the most important factors affecting agricultural production. Their control is carried out using herbicides that affect environmental pollution. The reduction in the use of herbicides has so far mainly been reduced to compliance with the recommended doses of application as well as to the harmonization of their application with the measures of mechanical weed control. Today, there is a trend to develop smart systems that first perform precise weed detection. The collected data is then precisely processed. In the end, a precise application of herbicide in a certain amount is carried out only in those places where there are weed plants. The goal is to apply the minimum amount of herbicide, in the right way in the right place, and at the right time (Šćepanović et al., 2018, Wu et al., 2021).

The prerequisite for choosing the most effective herbicide or their combination is the determination of the weed flora in the field. The correct determination of weeds is particularly important in the earlier stages of development, that is, in the stage of development of cotyledons and the first leaves, because the application of herbicides in these stages enables the use of lower concentrations. In this way, a reduction in the amount of herbicides introduced into the environment is achieved.

The correct determination of weeds is the basis for the application of the DSS (decision support system) model, and the weed germination forecast model, which indicates to the producer which herbicide, in what dose, and at what time, should be applied.

Determining weeds in the stage of cotyledons and first leaves is the most difficult to do in practice. This is especially demanding with monocotyledonous

weed species (grasses) which are morphologically very similar. In order to make the determination of weed species easier and faster for agricultural producers, various applications are being developed for the automatic digital recognition of weed species. The idea of these applications is to provide the agricultural producer with the help of modern technology (ordinary smartphones) information about which weed species dominate the field.

Various morphological characteristics of plants are used to identify weeds, such as the shape of cotyledons and leaves, color, surface structure and texture, shape of edges and leaf surfaces. The algorithm compares such information with features "learned" from the photos used to create the algorithm. As an output, the model suggests a series of species for which the algorithm has calculated a high similarity to the photographs. The result is transmitted back to the user's device (smartphone) and provides information about the weed species represented. Until now, only applications have been developed for the determination of dicotyledonous weed species, which is expected due to the high level of difficulty in recognizing monocotyledonous species (Šćepanović et al., 2018).

Santel i sur. (2018) state that such applications for some weed species that have specific leaves gave very high precision of identification, while for species that have more complex and denser leaves, it is less reliable.

According to the results of Razfar et al. (2022) using a deep learning model can positively influence the efficiency, application time, and total soybean production with high accuracy.

The mentioned models can be of special interest in the Republic of Serbia and similar countries. Namely, our conditions are characterized by a large number of small, fragmented individual farms that do not have direct contact with experts in the field of plant protection. In such conditions, this technology facilitates the detection of conditions on the ground, and on the basis of this data, it is easier to find the right solutions that should be applied in weed control in accordance with current trends.

Conclusion

The main aims of modern plant protection are the optimal intake of resources while maintaining high yields and crop quality and reducing environmental pollution and degradation. In order to succeed in this, one of the solutions is to reduce the application of herbicides to a minimum while maintaining high efficiency with the help of precision agriculture. Robotic weed

control with targeted herbicide application can reduce the use of herbicides by up to 90%. Choosing the right herbicide, time, and place of application can be facilitated by new technologies for determining weed species such as deep learning models. These models can then be transferred to smaller field devices such as the Jetson Nano suitable for image processing.

Acknowledgement

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NPK FERTILIZER ADDITION EFFECT ON NARDUS STRICTA TYPE GRASSLAND IN KOPAONIK MOUNTINE

Vladimir Zornić[1], Mirjana Petrović[1], Snežana Babić[1], Đorđe Lazarević[1], Vesna Đurović[2], Dejan Sokolović[1], Dalibor Tomić[2]

Abstract: This study examines how the addition of NPK fertilizers impacts the change in *Nardus stricta* type grasslands. The investigation was carried out on the mountain Kopaonik between 2009 and 2012. The effects of four fertilizing treatments: control; N60 (N60P60K60); N90 (N90P60K60); N120 (N120P60K60) on plant species composition, Shannon evenness, and E_{GQ} (evaluation grassland quality index) were researched. The control treatment was dominated by *Nardus stricta* species, while *Festuca rubra* and *Agrostis capillaris* presence was a feature of fertilizing treatments. The highest nitrogen intake (N120) had Shannon evenness that was very similar to that of the control. All fertilizing treatments resulted in increasing forage value.

Keywords: grassland, *Nardus stricta*, Shannon evenness, forage value

Introduction

The *Nardus stricta* type grasslands are widespread nearly all over the world (Trivedi et al., 2008). In the Balkans, those communities are widespread, especially in mountainous areas. It was mainly created as a result of hundreds of years of regular and low intensity grazing or grazing-hay management (Korzeniak, 2016). High-mountain *Nardus stricta* grasslands are exceptions and regarded as almost climax forms (Galvanék and Janák, 2008). *Nardus stricta* grassland is a uniform community dominated by *Nardus stricta* species (40 to 60%) (Alibegović Grbić et al., 2008).

Fertilization is a key management factor and a prerequisite for successful biomass production in grasslands. Application of mineral fertilizers, especially N, increases herbage yield quickly and changes a grassland vegetation structure (Smits et al., 2008). Addition of N resulted in increase of yield, which is caused by increasing presence of grasses as more productive and stronger competitive species in comparison to others (Wesche et al., 2012). Because of growing

[1]Institute for forage crops Kruševac, 37251 Globoder, Serbia (vladimir.zornic@ikbks.com)
[2]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia

asymmetry, grasslands have been transformed into homogeneous swards of a few more productive and higher-quality grass species (Zornić et al., 2022).

In this context, the study's objective was to determine how mineral fertilizer s affected the compostion of plants, Shannon evenness, and the quality of the grasslands of the *Nardus stricta* type.

Materials and methods

Study site The field experiment was set up at a height of 1550 meters above sea level in Rendara, Serbia, on the mountain Kopaonik. The research was carried out over a four-year period (2009 – 2012). Grassland community was classified as *Nardus stricta* type grassland.

Soil and weather conditions. The soil type at the experimental site was a rendzina. The soil pH (KCl) 3.99, plant-available phosphorus (P) content was 3.1 mg/100 g soil, potassium (K) was 12.9 mg/100 g soil and total humus content was 9.9%.

Experiment. The experiment was established in March 2009, as randomized block design with four replications. Each plot was 4 m × 5 m in size. Four NPK fertilizer treatments were applied: control (unfertilized); N60 (N60P60K60); N90 (N90P60K60); N120 (N120P60K60). The following fertilizers were used: NPK 15:15:15 and calcium ammonium nitrate fertilizer (KAN - 27% N).

Botanical analyses Before mowing, average samples of biomass were collected from each treatment. All plant species in these samples were separated by hand and measured. The biomass proportion of each species was calculated.

Shannon evenness J', was calculated using the data number of species S and Shannon diversity index H': $J' = H'/\ln S$. The Shannon diversity index H' equals to:

$$H' = -\sum_{i=1}^{S} (p_i \times \ln p_i)$$

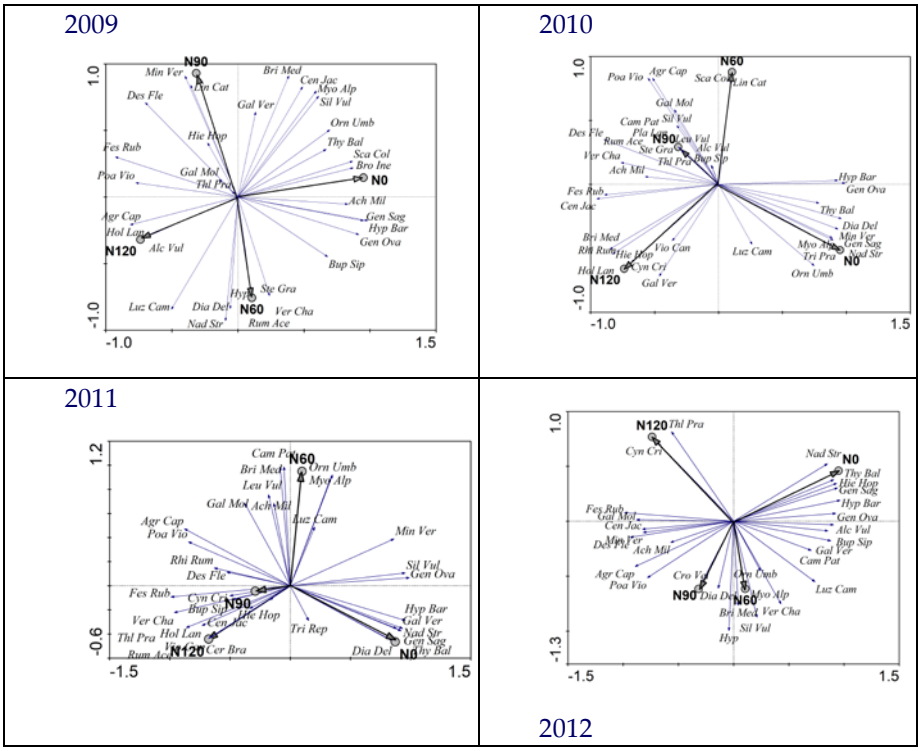
Where pi is proportion of i th species in sward.

Individual species forage value (FV) and abundance of species D (%) were used to evaluate grassland quality (EGQ) (Novak, 2004). $E_{GQ} = \Sigma(DxFV)/8$

The influence of the treatments, NPK application and years of investigation at Shannon evenness and EGQ were tested by using main effect ANOVA. Significant differences between the treatments were analyzed by Fisher's LSD test. Species composition change caused by NPK fertilizers was analyzed by direct gradient RDA (redundancy analysis) CANOCO in Windows 4.5 package.

Results and discussion

Based on the RDA analysis (Graph 1), fertilizer treatments affected the percentage of individual plant species on a sward. The effect of fertilizers is related to the studied year. Fertilization promoted grass species such as *Agrostis capillaris*, *Festuca rubra*, *Deshampsia flexuosa* and *Poa violacea*, in 2010, 2011 and 2012 year.



Graph 1. Ordination diagram of the results of RDA (redundancy analysis) plant species composition data in 2009, 2010, 2011 and 2012 on *Nardus stricta* type grassland, influenced by fertilizing. Species abbreviations: *Agr cap - Agrostis capillaris, Des fle - Deschampsia flexuosa, Fes rub - Festuca rubra, Nar str - Nardus stricta, Poa vio - Poa violacea.*

Nardus stricta had the highest percent in a treatment with no fertilization. The stronger effect of mineral fertilizers was observed as fertilization was applied for a longer period of time. According to an investigation by Stošić and Lazarević (2007), applications of mineral fertilizers in *Nardus stricta* grassland

primarily changed it into an *Agrostis capillaris* and *Festuca rubra* community. The results confirm this hypothesis. Even though those species had always been a less frequent part of swards, their percentage increased in response to the addition of N (Mountford et al., 1996). In fertilized treatments, Samuil et al. (2018), found a decrease in *Nardus stricta* presence and an increase in productive *Arrenatherum elatius* and *Festuca rubra* grassland types.

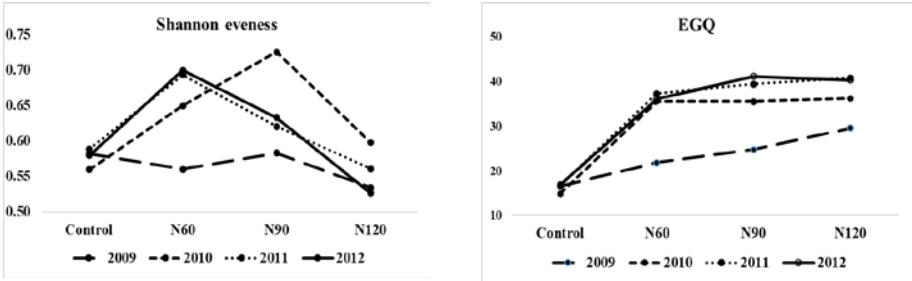
Shannon evenness increased in treatments N60 and N90, based on the main effect ANOVA (Table 1). The Shannon evenness in treatment N120 was very similar to that of the control. There were no statistically significant differences between research years. After four fertilizing years, treatment N120 had the lowest diversity evenness (Graph 2). Increased soil fertility status increased the percentage presence of species with higher soil nutrient requirements, and thus these species spread on fertilized plots (Samuil et al., 2013).

Table 1. Main effect ANOVA results of influence NPK fertilizers on Shannon evenness and E_{GQ} in *Nardus stricta* type grassland

Tretman	Shannon evenness	E_{GQ}
Control	0.56ab	16.2b
N60	0.65a	32.7a
N90	0.64a	35.2a
N120	0.56b	36.7a
Investigation years		
2009	0.56a	23.2b
2010	0.63a	30.5a
2011	0.62a	33.5a
2012	0.61a	33.5a

Values followed by different letters are significantly different ($p<0.05$) according to the LSD test;

According to Novak (2004), the obtained results classify the initial community into worthless communities based on the quality index. Due to fertilizing and years of research, there was a significant increase in E_{GQ}. (Table 1). Higher E_{GQ} in fertilized plots than control was recorded, during the whole experimental period (Graph 2). Average E_{GQ} in 2010, 2011 and 2012 were significantly higher than in 2009. Application NPK fertuilizers increased grassland quality in grassland comunity *Festuco nardetum subalpinum* in the same area (Zornić et al., 2022). Higher forage values in fertilizing treatments were consistent with the results obtained by Pittarello et al. (2018).



Graph 2. Shannon evenness and E_{GQ} in investigate *Nardus stricta* type grassland during four years fertilizers addition

In general, EGQ and Shannon evenness increased in treatments N60 and N90. In treatments N120, forage values increased while the diversity index remained unchanged when compared to the control.

Conclusion

During the four-year experiment period, significant differences in plant species composition occurred as a result of NPK fertilizer addition. In comparison to low productive *Nardus sticta* species, more productive *Festuca rubra* and *Agrostis capillaris* grasses were stimulated by fertilizer application over a four-year period.

Shannon evenness increased as a result of the NPK fertilizers N60P60K60 and N90P60K60. In terms of grassland diversity, there were no differences between N120P60K60 and the control. The value of forage was significantly increased in NPK treatments.

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THE ROLE OF COBALT IN FORAGE LEGUMES

Dalibor Tomić[1], Vladeta Stevović[1], Milomirka Madić[1], Miloš Marjanović[1], Nenad Pavlović[1], Đorđe Lazarević[2], Mirjana Petrović[2], Vladimir Zornić[2], Jasmina Knežević[3]

Abstract: The growth and metabolism of plants, especially on acidic soils, largely depend on the concentration of cobalt (Co) in the soil, i.e. the rhizosphere. An optimal supply of cobalt is essential for N_2 fixation of Rhizobium bacteria that are in symbiotic relationships with leguminous plants, influencing their better growth and supplying them with nitrogen. When there is a lack of Co in the plant, the organic production of legumes falls. Indirectly or directly, Co also affects other metabolic processes in plants. The aim of the work was to analyze the importance of optimal provision of forage legumes with cobalt for obtaining high and quality yields of forage and seeds.

Keywords: cobalt, forage legumes, mineral nutrition

Introduction

Cobalt is not classified in the group of essential elements for plants, but it is in the group of useful elements (Bakkaus et al., 2005). The normal concentration of cobalt in the dry matter of plants is low and ranges from 0.1-10 μg g^{-1}. The distribution of cobalt in plants depends on the plant species, and it is generally highest in the leaf. The concentration of cobalt in the leaf of forage plants varies from 0.6-3.5 ppm. It is most abundant in legumes (Palit et al., 1994), for which it is particularly important for the process of nitrogen fixation. The distribution of cobalt in plant organs also depends on the stage of plant development. In the early phase of plant development, large amounts of cobalt are absorbed in leaves and stems (Kenesarina, 1972), while before flowering and until maturation, the largest amounts are found in nodules. Plant organs contain cobalt in the following ascending order: roots, leaves, seeds, stems.

[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia (dalibort@kg.ac.rs).
[2] Institute for Forage Crops, Globoder bb., 37251, Serbia.
[3] University of Priština, Faculty of Agriculture, Kopaonička, 38219, Lešak, Serbia.

According to Palit et al. (1994) the growth and metabolism of plants, especially on acidic soils, depend to a large extent on the concentration of cobalt in the soil, i.e. the rhizosphere.

Cobalt affects plant metabolism and growth and is an essential component of many enzymes and coenzymes. According to Kobayashi and Shimizu (1999), cobalt is used in a limited number of enzymes, unlike iron and zinc. However, there are several enzymes that are activated by cobalt ions: E. coli acetylornithinase, Methanobacterium thermoautotrophicum cyclic 2,3-diphosphoglycerate hydrolase, α-d-mannosidase. Cobalt was not found in enzymes that enter the respiratory chain.

Cobalt is also important for normal leaf development, inhibition of ethylene biosynthesis and stimulation of alkaloid biosynthesis (Farooq et al., 2012). It is a component of vitamin B_{12} and cobamide coenzyme and thus helps in the process of fixing molecular nitrogen in the root nodules of legumes (Tomić, 2017). The positive effects of cobalt are reflected in slowing down the aging of leaves, increasing resistance to drought, regulating the accumulation of alkaloids in medicinal plants, inhibiting ethylene biosynthesis (Palit et al., 1994). Indirectly or directly, cobalt affects the metabolic processes of plants, such as: lipid metabolism, auxin turnover, photochemical activity of chloroplasts, etc. (Petrović and Kastori, 1992).

The aim of the work was to analyze the importance of optimal provision of forage legumes with cobalt for obtaining high and quality yields of forage and seeds.

Cobalt in soil

The total concentration of Co in the soil is 1-40 mg kg^{-1}. It includes Co associated with insoluble minerals or within stable crystal structures, which is not available to plants. According to Collins and Kinsella (2011), pedogenetic factors including soil pH value, concentration of total, extractable, isotopic and water-soluble cobalt can be found in specific correlations with cobalt content in plants. Therefore, understanding the factors that influence cobalt uptake is necessary for continuous plant production as well as for the possibility of remediation of contaminated systems. Kukier et al. (2004) found a significant positive correlation between soil pH and plant cobalt content. Contrary to this, a large number of studies indicate a negative correlation between soil pH and cobalt uptake by plants (Kukier et al., 2004; Faucon et al., 2009). This means that, in general, a universal relationship between soil pH and cobalt uptake cannot be concluded, but that it depends on many factors, most of all on the

type and properties of the soil. Lee et al. (2004) indicate the existence of a significant negative correlation between soil iron content and cobalt uptake. A higher concentration of manganese can negatively affect cobalt uptake (Faucon et al., 2009). According to McKenzie (1972), high amounts of manganese and humus in the soil inhibit cobalt uptake. Most of the cobalt in the soil is fixed in this way and is therefore unavailable to plants. Collins and Kinsela (2010) indicate the importance of the influence of soil solution composition on cobalt uptake. Numerous studies indicate that with an increase in the concentration of easily soluble cobalt in water, its absorption from the soil also increases (Li et al., 2009).

When cobalt is added to the soil, geochemical reactions often result in its redistribution into the solid phase of the soil, so that there is no increase in the concentration of its easily soluble form (Wendling et al., 2009). This is also confirmed by Sherrell (1990), according to which the annual application of 0.09 mg kg^{-1} cobalt for nine years on loamy soil (pH 5.6) did not significantly increase the concentration of cobalt in white clover and alfalfa. Therefore, and considering the low mobility of cobalt in the plant (Austenfeld 1979) and its faster movement from the aerial part to the root compared to the opposite direction (Palit et al., 1994), foliar nutrition in relation to soil fertilization more effectively establishes its optimal status in plants. The same authors indicate that the movement of cobalt through the plant is faster in the descending path than in the opposite direction.

The role of cobalt in nitrogen fixation

Optimum provision of plants with cobalt contributes to enhanced nitrogen fixation in all Rhizobium species, and thus to the growth of legumes (Collins and Kinsela 2011). Cobalt is the central atom in the porphyrin structure of the coenzyme cobalamin and is essential for nodulation and development of bacteroids. Cobalt is a component of vitamin B_{12}, which is included in the composition of enzymes and coenzymes that participate in the process of nitrogen fixation in leguminous nodules (Mathur et al., 2006). Various studies indicate that vigor, nodule development and nitrogen content of legumes depend on the cobalt content of the soil. According to Vukadinović and Lončarić (1997), cobalt is a necessary element for symbiotic nitrogen-fixing microorganisms. When there is a lack of Co in the plant, the organic production of legumes falls. Das (2000) indicated that there are three specific cobalamin-dependent enzyme systems in rhizobium that can be attributed to the effect of

cobalt on nodulation, ribonucleotide reductase and methylmalonyl coenzyme A mutase.

The application of cobalt in very small amounts to the aerial parts of plants or in the soil had a positive effect on symbiotic nitrogen fixation (Tomić et al., 2015a), yield components and seed yield in red clover (Tomić, 2017).

Powrie (1964) in alfalfa (*Medicago sativa* L.) and Ozanne et al. (1963) in subterranean clover (*Trifolium subterraneum* L.) determined that a good supply of soil Co influenced a significant increase in plant mass and an increase in the amount of fixed nitrogen.

Dilworth et al. (1979) state that the number of bacteroids and leghemoglobin content in nodules of blue lupine (*Lupinus angustifolius* L.) directly depends on the cobalamin content, and nitrogen fixation activity is dependent on both the cobalt status and the cobalamin content. The authors also conclude that lupine crown nodulation is significantly reduced under cobalt deficiency, most likely as a consequence of reduced nodule initiation and that normal nitrogenase activity cannot occur below the critical concentration of cobalt in nodules.

Pattanayak et al. (2000) state that the application of cobalt in a concentration of 250 mg kg^{-1} and 500 mg kg^{-1} of soil in cowpea (*Vigna sinensis* L.) influenced a significant increase in the number of nodules per plant, the number of effective nodules per plant, the mass of effective nodules per plants, accumulation of dry matter in plants, number of pods per plant and seed yield per hectare. The authors state that cobalt is an essential element for nodulation and fixation of atmospheric nitrogen by leguminous plants.

Adding cobalt to the soil during forage pea production, in the amount of 10-20 g ha^{-1} in the form of cobalt chloride, especially in combination with nitrogen fertilization, had an effect on increasing seed germination, leaf chlorophyll content, shoot length, root length, dry matter content matter in shoots and roots, nodules number per plant, flowers number per plant, pods number per plant, thousand seed weight, seed yield, nitrogen content in shoots and stem and cobalt content in nodules and shoots (Acbar et al., 2013).

Cobalt in the amount of 0.16 mg g^{-1} of soil influenced a significant increase in the number and mass of nodules, nitrogen concentration in nodules, leghemoglobin content, total biomass production, seed yield compared to untreated peanut plants (*Arachis hypogaea*) (Nadia et al., 2012).

According to the results of Jayakumar and Jallel (2009), foliar application of cobalt or its application over the soil had a positive effect on better nodulation, better growth, increase in plant dry matter yield and soybean seed yield.

Other roles of cobalt in forage legumes

Cobalt is important for the normal development of a number of physiological reactions in the process of photosynthesis (Lipskaya, 1972). In lower concentrations, cobalt has a positive effect on the Hill reaction with a simultaneous decrease in the amount of chlorophyll and an increase in the number of chloroplasts per unit of leaf area. The increase in photosynthesis activity can be associated with the redistribution of pigments (increase in the amount of chlorophyll b and decrease in the amount of chlorophyll a), or direct entry into the light and dark phases of Hill's reaction. The positive impact of the provision of Co to plants is manifested through increased chlorophyll content (Palit et al., 1994), greater thickness of the palisade tissue, increased number and size of chloroplasts (Lipskaya, 1972). Cobalt is an important element for the photosynthesis process in perennial legumes. The addition of cobalt to the nutrient solution at a concentration of 1 ppm in the form of $CoCl_2$ in soybeans led to an increase in the content of chlorophyll and vitamin B_{12} (Ahmed and Evans, 1960).

Cobalt plays an important role in the respiration process in leguminous plants, as well as in the regulation of transpiration because it affects the work of stomatal cells (Rauser and Dumbroff, 1981). According to Zeid (2001), the presence of cobalt in the nutrient solution is very important for seed germination and achieving high plant yields.

Delwiche et al. (1960) applied 0.1 μmol L^{-1} Co over the soil with the addition of Rhizobium inoculum in alfalfa and determined that there was a significant increase in green mass yield in comparison to the control. Also independently applied cobalt ions or Rhizobium inoculation, had an effect on higher plant growth. The nodules that were on treatment with the application of cobalt had a higher capacity for nitrogen fixation than those on the control variant, which was determined by the application of isotopic nitrogen N^{15} in the substrate.

Reith and Burridge (1983) applied 0.5 kg ha^{-1} of cobalt in the form of cobalt-sulfate and found an increase in the content of cobalt in the forage of several types of clover, to a greater extent on peat than on mineral soils.

The foliar addition of cobalt had in general a positive effect on seed yield and yield components in all cultivars of red clover (Tomić et al., 2014). The higher yield on the variant with cobalt is the consequence of the significant increase in flower number per inflorescence, i.e. seed number per inflorescence. Foliar treatment with cobalt achieved a positive impact on forage yield of the

cultivars of red clover (Tomić et al., 2015b). The hay yield results are consistent with the results of forage yield.

Toxic effect of cobalt in plants

The boundary between useful and toxic concentrations of cobalt in forage plants is narrow. A high concentration of cobalt in plants is undesirable and can adversely affect its physiological and biochemical functions (El-Sheejh et al., 2003). In interaction with other elements, cobalt forms complexes. The cytotoxic and phytotoxic activity of these complexes depends on their physical and chemical properties (Palit et al., 1994). Competitive absorption and mutual activation of related metals are factors on which the effect of cobalt in reactions depends. Toxic concentrations of cobalt inhibit active ion transport in higher plants. Cobalt participates in the formation of chlorophyll b, but causes damage to plastids and affects the change in the structure and number of chloroplasts per unit area in the leaf. Its harmful effect when it is in excess is reflected in inhibiting the activity of PS2 and reducing the export of photoassimilates in the dark phase of photosynthesis. In C_4 plants, cobalt interferes with carbon dioxide fixation by inhibiting the activity of enzymes involved in that process. Cobalt slows down the processes of cytokinesis and karyokinesis. Excess cobalt hinders the synthesis of RNA and DNA, most likely by modifying a large number of endonucleases and exonucleases.

The toxic effect of cobalt on the morphology of plants manifests itself in the form of chlorosis, leaf fall, pale leaf nervure and reduced plant growth (Palit et al., 1994).

Foliar application of cobalt affected a significant reduction of the chlorophyll content in the leaves of all cultivars of red clover compared to the control variant without application of cobalt (Tomić et al., 2015c). The reason for this is the harmful effect of cobalt applied in larger quantities.

Conclusion

The concentration of cobalt in soils is 1-40 mg kg^{-1}, and in the dry matter of plants 0.1-10 μg g^{-1}. When cobalt is added to the soil, geochemical reactions often result in its redistribution in the soil, so that there is no increase in the concentration of its easily soluble form. In general, a universal relationship between soil pH value and cobalt uptake cannot be concluded, but that it depends on many factors, most of all on the type and properties of the soil. A significant negative correlation exists between the content of iron and

manganese and the concentration of easily accessible cobalt in the soil. Due to all of the above, given the low mobility of cobalt in the plant and its faster movement from the above-ground part to the root compared to the opposite direction, foliar nutrition in relation to soil fertilization more effectively establishes its optimal status in plants.

Cobalt is included in the structure of vitamin B_{12}, which is an integral part of enzymes and coenzymes, important for the process of nitrogen fixation in all species of Rhizobium. An optimal supply of cobalt has a positive effect on growth and development, and thus on the forage yield and seeds of a large number of forage legumes such as: red clover, alfalfa, cowpea, subterranean clover, lupine, peas, peanuts, soybeans. The positive impact is reflected in the increase of nodules number per plant, effective nodules number per plant, effective nodules mass per plant, the accumulation of dry matter in plants, greater plant height, root length, an increase in the pods number per plant, flowers number per plant, pods number per plants, thousand seeds weight, content of chlorophyll in the leaf and others. In addition to the above, the optimal presence of cobalt in forage legumes has a positive effect on the intensity of the photosynthesis process, seed germination and vigor, yield components and seed yield.

A high concentration of cobalt in leguminous plants is undesirable because it can adversely affect physiological and biochemical functions in the plant, and the border between useful and toxic concentrations of cobalt is very narrow.

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GRAIN YIELD OF MAIZE HYBRIDS IN DIFFERENT LOCATIONS IN CENTRAL SERBIA

Milomirka Madić[1], Dalibor Tomić[1], Aleksandar Paunović[1], Vladeta Stevović[1], Milan Biberdžić[2], Dragan Đurović[1], Miloš Marjanović[1]

Abstract: Field trials with 11 maize hybrids (9 newly released and two standards) of the FAO maturity groups 300-600 were sown in four locations. The aim of the work was to recommend hybrids for individual location based on their reaction to different agroecological conditions, as well as to evaluate the possibility of replacing later hybrids with hybrids of earlier maturity groups, with certain changes in agrotechnics. ZP hybrids of the latest generation showed high grain yield potential and stability, as well as wide adaptability to different ecological conditions, degree of soil fertility and application of agrotechnical measures. A significantly higher grain yield of all hybrids compared to other locations was at the Valjevo location, that is, the location with the highest amount and favorable distribution of precipitation during the growing season. In these trials, maize hybrids of different FAO maturity groups were grown with the same number of plants per unit area. For maize hybrids with shorter vegetation, which are characterized by a lower plant height, a greater number of plants per unit area is recommended, compared to hybrids with a longer growing season. With changes in agrotechnics in this direction, along with the advantages of earlier hybrids, which are seen in avoiding critical periods for water, the grain yield of earliy hybrids could reach the yield level of later hybrids.

Keywords: maize, hybrid, grain yield, location

Introduction

World crop production increased by 52% between 2000 and 2020 (FAOSTAT 2021). Cereals were the main group of crops, where corn production grew three times faster than wheat, so in terms of total production, corn became the second crop in the world (Grčak et al. 2020). The increase in production can mainly be attributed to the cultivation of high-yielding varieties, i.e. hybrids, as well as the increase in the use of fertilizers, pesticides, irrigation and, to a lesser

[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia (mmadic@kg.ac.rs)
[2]Faculty of Agriculture, Lešak, University of Priština, (Kosovska Mitrovica) Serbia

extent, the increase in arable land. In 2022, the average grain yield of maize in the Republic of Serbia was about 4.8 t ha^{-1}, which led to a decrease in total maize production by 3.3 million tons compared to 2020. In the last 25 years, the grain yield of maize depends more on meteorological conditions during the growing season, which is often characterized by the occurrence of "extreme climatic events" (Bekavac et al., 2010, Pavlov et al., 2011). The most important factors recently leading to the reduction and variability of yields in most regions are the increase in temperature and uneven distribution of precipitation, especially in critical periods of maize growth (Kovačević et al., 2010; Stojaković et al., 2010; Arunkumar et al., 2020; Madić et al., 2021). The high potential for grain yield of maize hybrid is best manifested in conditions when the plants are supplied with 550-700 mm of water precipitation during the growing season (Filipović et al., 2015). One of the ways to achieve a compromise, and satisfy the interests of both producers and breeders, is to divide the cultivation area of a crop in the region based on the geographical, climatic and soil conditions that characterize them, and recommend hybrids for each region (Babić et al., 2013). In the Republic of Serbia, a large number of maize hybrids have been created and relaised so far, and it is necessary to set up trials every year in a large number of locations, with the aim of informing the producers to the newly selected hybrids, and to check the new hybrids in different agroecological conditions.

Taking into account the frequent reaction of hybrids to changing agroecological conditions, four locations were selected for these experiments. The aim of this research was to select the most suitable hybrids for a specific location, based on their reaction to the agroecological conditions of the location. In addition, the grain yield of hybrids of different maturity groups would indicate the possibility of replacing hybrids with longer vegetation with earlier hybrids, with an increase in the number of plants per unit area. By choosing such hybrids, the consequences of climate change would be mitigated, that is, the influence of high temperatures and drought in critical periods of maize growth would be avoided.

Materials and methods

Nine perspective SC hybrids of maize FAO maturity groups 300-600 (ZP 341, ZP 427, ZP 443, ZP 548, ZP 555, ZP 560, ZP 600, ZP 606, ZP 666) and two standards (ZP 434 and ZP 677) were selected for this experiment.The experiment was set up in 2018 at four locations, on soil of different production characteristic: Divci (Valjevo, 44017'58" N, 20001'02"N, 148 m a.s.l., alluvium

soil), Lađevci (Kraljevo 43091'05'' N, 20035'12'' N, 209 m a.s.l., smonica soil), Topola (44015'27'' N, 20048'28'' S, 140 m a.s.l., alluvial soil), Aleksandrovac (Smederevska Palanka 44027'06'' N, 21011'51'' S, 85 m a.s.l., valley-meadow land). In the first part of April, 8 rows of each hybrid on a 200 m length were sown in all locations. Sowing was done at a between-row distance of 0.7 m, and a within-row distance of 0.25 m, so that all hybrids were grown at a density of 51,143 plants per hectare. During the growing season, the usual production technology was applied along with basic cultivation: 20 t ha^{-1} of manure was added to the soil; in pre-sowing preparation 400 kg NPK ha^{-1} (16 : 16 : 16) and in phase 5-6 leaves 250 kg ha^{-1} KAN. As part of the protection measures, herbicides were used to control weeds: Mont 0.75 5 l ha^{-1} + Terbis 0.5 l ha^{-1}. In all locations crops were not irrigated. At maturity, the yield of cobs from 100 plants from 4 inner rows was measured from each plot, on the parts where sprouting was uniform and converted to grain yield t ha^{-1} with 14% moisture. The obtained data were processed by the two-factor analysis of variance (hybrid, location) without repetition, with a preliminary check of additivity (absence of interaction between factors) by Tukey's test (Tukey, 1949). For factors for which the F test was statistically significant, a comparison of individual values were performed using the LSD test.

Weather conditions

Average annual air temperatures in 2018 did not differ much by location (Table 1). However, if comparing individual months, some differences can be noticed. Thus, the locations of Valjevo and Kraljevo had slightly warmer February and March, compared to the locations of Topola and S. Palanka, which had a warmer May compared to the other locations. In June, July and September, similar mean monthly temperatures were measured in all locations, but the Topola location had a slightly lower temperature compared to the other locations. The highest mean monthly temperatures in all locations were recorded in August when the precipitation deficit was also pronounced. There was a similar trend in the amount of precipitation in all locations, except in the area of Topola, where a slight increase in the amount of precipitation was recorded in July compared to June (Table 1), while in other locations a decrease in the monthly amount of precipitation in June compared to July was observed.

Observing the sums of precipitation in the period of intensive maize growth (May, June and July), it can be seen that it was higher in the locations of Valjevo and Kraljevo compared to the locations of Topola and S. Palanka (Table 1). In all locations, the most rainfall during the growing season was in May, and the least in August. The amount of precipitation in May was 140.4 mm in the Valjevo,

which is 18 mm more compared to the Kraljevo, i.e. 38.1 mm more compared to the S. Palanka, while the Topola location recorded two times less rainfall (70.9 mm).

Table 1. Mean monthly temperatures (T°C) and precipitation (mm) in 2018

Monthly	Valjevo		Kraljevo		Topola		S. Palanka	
	T°C	mm	T°C	mm	T°C	mm	T°C	mm
IV	13.7	25	13.7	69.9	13.3	41.2	13.3	36.7
V	17.6	144	17.8	122.4	18.	70.9	18.0	102.3
VI	20.5	63.9	19.0	90.1	19.9	56.5	20.0	78.3
VII	22.6	42.7	22.2	23.2	21.6	60.6	22.2	7.4
VIII	23.7	28	23.8	23.0	23.1	50.1	24.0	31
IX	16.4	54.7	16.2	48.6	16.1	49.6	15.9	52.1
Average/ Sum	19.1	343	18.9	377.2	18.7	328.9	18.9	307.8

Results and discussion

The latest generation of maize hybrids have the potential for grain yield of 15 to 20 t ha^{-1}. However, the realized grain yields in our conditions are around 6.5 t ha^{-1} in average years, i.e. in the most productive years up to 8.0 t ha^{-1}, which indicates that only 25-30% of the grain yield potential is realized (Filipović et al. 2015). Starčević et al. (1991) stated that in years with favorable weather conditions, the difference in grain yield is greater in favor of hybrids with a longer vegetation period (from 18 to 26%), in less favorable years, the yields are even, while in unfavorable years, early hybrids had a higher grain yield (up to 7%) compared to mid-early and mid-late hybrids. The yield and water content in the grain were significantly dependent on weather conditions in the growing season (Jagła et al. 2019). Considering the amount and distribution of rainfall in 2018, it can be seen that it was relatively favorable for corn production. Observed as a whole, the grain yield ranged from 5.91 t ha^{-1} for hybrid ZP 443 at the S. Palanka location to 10.37 t ha^{-1} for hybrid ZP 600 at the Valjevo location (Table 2). At the Valjevo location, all hybrids had a significantly higher grain yield and a higher grain water content compared to the other locations. The lowest grain yield, on average for all hybrids, was at the Kraljevo location, while the lowest water content in the grain was at the S. Palanlka. The hybrids ZP 600, ZP 548 and ZP 560 had a grain yield of over 10 t ha^{-1}, while the hybrid ZP 443 had the lowest yield, unexpectedly low at the S. Palanka location, which is the most likely the result of some uncontrollable

factor. The ZP 666 achieved the highest grain yield at the S. Palanka which, compared to other locations, was characterized by the lowest amount of precipitation during the growing season.

Table 2. Grain yield (t ha^{-1}) and grain moisture (%) of maize hybrids in four locations

	Locations							
Hybrids	Valjevo		Kraljevo		Topola		S. Palanka	
	Grain yield	Grain moisture	Grain yield	Grain moisture	Grain yield	Grain moisture	Grain yield	Grain moisture
ZP 341	9.25	17.60	6.85	16.00	7.82	15.10	6.47	12.30
ZP 427	8.34	17.60	6.32	17.40	7.49	13.50	6.96	12.60
ZP 443	9.06	18.30	7.29	17.00	8.54	13.60	5.91	13.10
ZP 548	10.03	18.10	7.39	17.00	8.52	13.40	7.07	13.70
ZP 555	8.71	19.70	6.57	17.60	8.09	16.80	7.28	14.10
ZP 560	10.01	19.70	6.87	18.60	7.49	17.50	7.55	13.90
ZP 600	10.37	19.50	7.04	18.00	7.79	16.40	8.02	14.70
ZP 606	9.60	19.30	7.19	17.00	7.75	15.90	8.81	15.60
ZP 666	8.53	19.70	6.94	17.80	6.88	15.20	9.23	15.30
St 1	10.29	16.40	8.02	16.00	6.84	14.30	7.46	13.20
St 2	9.51	18.40	7.03	15.80	5.94	13.60	6.88	14.20
Average	9.43	18.57	7.05	17.11	7.56	15.03	7.42	13.88

Two standard hybrids for grain production (ZP 434 and ZP 677) were also included in the experiment. Both standards had a higher yield at the Valjevo location, while the lowest yield was recorded at the S. Palanka. In years with a small amount of precipitation, hybrids with a shorter growing season had a higher average grain yield, while in years with average and above-average rainfall, higher yields were achieved with hybrids with a longer growing season (Branković - Radojčić et al. 2017). Changes in the environment affect the growth and yield grain due to significant genotype × environment interactions (GEI) (Changizi et al., 2014; Djurović et al., 2014; Stojaković et al., 2015; Madić et al., 2022). The obtained results indicate that the amount and distribution of precipitation, as well as average daily temperatures, greatly influenced grain yield and grain water content, which is in agreement with the results of Petrovic et al. (2023). Analysis of the variance of grain yield indicates the existence of significant differences between locations, while differences between hybrids within locations were not significant (Table 2). On the other hand, in their research, Faria et al. (2017), Arunkumar et al. (2020) and Madić et al. (2022)

pointed out significant differences among environments, hybrids, and their interactions. Hybrid ZP 606 achieved the highest grain yield, on average for all locations(8.34 t ha^{-1}), while hybrid ZP 427 had the lowest yield (7.28 t ha^{-1}). Observing the results of ZP hybrids by FAO maturity groups, it can be seen that the highest average yields were achieved by mid-late hybrids (FAO 600), where ZP 606 (8.34 t ha^{-1}) and ZP 600 (8.31 t ha^{-1}) hybrids stand out. In the group of mid-late hybrids (FAO 500), the highest yield was recorded in hybrid ZP 548 (8.25 t ha^{-1}). It should be also pointed out that the mid-early hybrids (FAO 300-400), especially ZP 443 (7.70 t ha^{-1}) and ZP 341 (7.60 t ha^{-1}), which are not behind the previous ones in terms of grain yield. This indicates their good adaptability to different agroecological conditions.

Table 3. Mean grain yield and grain moisture in maize hybrids

		Grain yield (t ha^{-1})	Grain moisture (%)
Hybrids	ZP 341	7.60	15.25 b
	ZP 427	7.28	15.27 b
	ZP 443	7.70	15.50 b
	ZP 548	8.25	15.55 b
	ZP 555	7.66	17.05 a
	ZP 560	7.98	17.42 a
	ZP 600	8.31	17.15 a
	ZP 606	8.34	16.95 a
	ZP 666	7.90	17.00 a
	Standrad 1	8.15	14.97 b
	Standard 2	7.34	15.50 b
Location	Valjevo	9.43 a	18.57 a
	Kraljevo	7.05 b	17.10 b
	Topola	7.56 b	15.02 c
	S. Palanka	7.42 b	13.88 d

Column values marked with different lowercase letters are significantly different (P<0.05) according to the LSD test.

Based on the analysis of the results of a macro-trials with 15 maize hybrids at 30 different locations on the territory of Serbia, Stojaković et al. (2010) state that the hybrids from the FAO maturity groups 500 and 600 had a higher yield and lower water content in the grain compared to the hybrids FAO 700, which leads to the conclusion that in Central Serbia the sum the temperatures is a determining factor in the choice of hybrids. The consequences of stress caused by unfavorable weather conditions could be mitigated by growing hybrids more resistant to drought and introducing minor changes in agrotechnics

(Kovačević et al. 2010). The results of these studies indicate a significant impact of climate change on the grain yield of maize, which points to the necessity of changes in agrotechnical, as well as the creation of maize hybrids with greater adaptability, especially to the stress caused by high temperatures and water deficit. Filipović et al. (2015) state that trends in modern maize breeding in the world lead to the creation of hybrids of modern architecture, i.e. plants with a lower habitus, upright position of the upper leaves, cobs with a larger number of grain rows, a smaller number of grains in a row, as well as the ability to quickly release water from the grain during the period of maturity.

Conclusion

ZP hybrids of the latest generation showed high grain yield potential and yield stability, i.e. wide adaptability to different environmental conditions, soil fertility level and applied agrotechnical measures. The significantly higher grain yield at the Valjevo location compared to the other locations, can be explained by the greater amount and more favorable distribution of precipitation during the growing season. In these trials, maize hybrids of different FAO maturity groups were grown with the same number of plants per unit area. For maize hybrids with a shorter growing season, which are characterized by a smaller habitus, a greater number of plants per unit area is recommended compared to later hybrids. By changing agrotechnics in this direction, with the advantages reflected in avoiding critical periods for water, the grain yield of hybrids with a shorter growing season could reach the yield level of later hybrids.

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FLOWERING PHENOPHASE OF SOME APRICOT (*P. armeniaca* L.) CULTIVARS DEPENDING ON AIR TEMPERATURE

Ivan Glišić[1], Radmila Ilić[1], Tomo Milošević[1], Gorica Paunović[1], Ivana Glišić[2], Zorica Radičević[3]

Abstract: The paper presents the three-year results of the effect of basic climatic parameters on the flowering of 12 apricot cultivars in conditions of Čačak. The significant differences in the beginning, progression and duration of flowering among years were determined. The beginning of flowering in 2020 was on March 12, in 2021 on March 1, and in 2022 on March 24. Flowering phenophase in one cultivar lasted between 6 and 9 days in 2022 and between 8 and 16 days in 2021. Differences in the beginning of flowering among cultivars were more pronounced in years with earlier flowering date.

Keywords: apricot, the beginning of flowering, climatic conditions

Introduction

The region of Čačak is very famous for apricot production. The hilly - mountainous area with an average altitude of 300 - 500 m a.s.l., specific climate and favorable agro-ecological conditions allow good results in apricot cultivation and relatively stable yields. Despite the favorable conditions in the mentioned area, due to the early flowering of apricots, there are significant differences in the course of the flowering phenophase among the cultivars, as well as large differences in different years. Flowering is one of the most important phenophases for all fruit species. In apricot, this phenophase is of particular importance because apricot is a fruit species that blooms very early. This is one of the main reasons for irregular fruiting in this fruit species (Szalay and Szabo, 1999). Due to the spring frosts during or after flowering in certain areas, fruit formation fails. One of the main reasons of the variation in apricot production in Serbia is the freezing of flowers or unopened flower buds due to low winter and spring temperatures (Miloševićet al., 2008; Radičević et al., 2011; Glišić et al., 2019).

[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia (glishoo@yahoo.com)
[2]Fruit Research Institut - Čačak, Kralja Petra I, 9, Čačak, Serbia
[3]Republic Hidrometeorological Service of Serbia, Kneza Višeslava 66, Belgrade, Serbia.

The aim of the work is to analyze effect of the climatic conditions (mainly air temperature) before and during the flowering period of 12 apricot cultivars in a three-year experiment.

Materials and methods

The experiment was conducted during 2020- 2022 in an apricot orchard in the village of Gornja Gorevnica, which is located about 10 km northwest of Čačak (43°53'N; 20°21'E; 390 m above sea level).

The experiment inculuded 12 apricot cultivars: 'Tsunami', 'Aurora', 'Wonder Cot', 'Spring Blush', 'Orange Red', 'Goldrich', 'Betinka', 'Hungary Best', 'Roxana', 'Farbaly', 'NS-4' and 'Zaklopačka Ruža'. The trees were grafted onto a Myrobalan rootstock (*Prunus cerasifera* Ehrh.) and planted at a distance of 6 × 3.5 m (480 trees ha^{-1}). Training system was cauldron canopy with 3-4 basic shoulder branches. Standard cultural practices were applied, except irrigation.

Based on data from the Monthly Bulletins, of the Republic Hydrometeorological Service of Serbia, for air temperatures on February and March 2020, 2021 and 2022 were analyzed. The deviation of the mean air temperature from the multi-year average was calculated using the percentile method[1] and displayed graphically.

The investigation included detected characteristics of phenological phase of flowering (beginning, duration and end of flowering). The phenophase of flowering was determined by recording the start date (when open 10% of flowers) and the end of flowering (when with 90% of flowers the crowns fall slips). The duration of flowering phenophase is expressed in days. The results are presented graphically.

Results and discussion

The beginning of apricot flowering depends on the sum of air temperatures in January, February and March, but also on soil temperature.

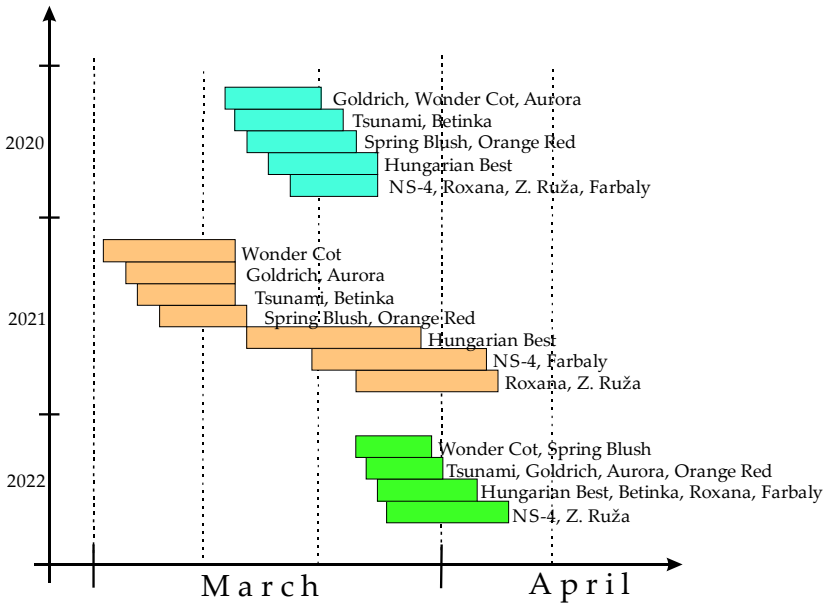
In 2020, February and March were significantly warmer than the long term average. The mean monthly temperature in February was 5.8°C, and in March was 7.6°C. February and March 2021 and 2022 were cooler than 2020 and had

[1] Then percentile of a quantity is that value of the observed quantity below which lies n percent of the data previously arranged in an ascending sequence.

similar mean monthly temperatures (4.10°C in February 2021and 4.05°C in February 2022; 5.05°C in March 2021 and 5.00°C in March 2022).

The beginning of apricot flowering in 2020, 2021 and 2022 was not quite in accordance with mentioned monthly temperatures. The earliest flowering was recorded in 2021, although the average monthly temperatures for February and March were lower in 2021 than in 2020.

The beginning and course of apricot flowering in 2020, 2021 and 2022 are shown in Graph 1.



Graph 1. Apricot flowering in 2020, 2021 and 2022

Flowering in 2020. The beginning of flowering in cultivars 'Goldrich', 'Wonder Cot', 'Aurora', 'Tsunami', 'Betinka', 'Orangered' and 'Spring Blush' was from March 12-15. 'Hungary Best' started flowering on March 16, and 'NS-4', 'Roxana', 'Zaklopačka Ruža' and 'Farbaly' on March 18. The end of flowering was in the period from March 18-26.

Differences in the beginning of flowering between cultivars were 6 days. The average duration of flowering for one cultivar was 7-8 days. The total duration of apricot flowering in 2020 (for all cultivars) was 14 days.

Flowering in 2021. Although the average daily temperature in February 2021 was lower than in 2020, flowering of early apricot cultivars began much earlier compared to the previous year. The order of cultivars by start of flowering was similar to 2020, with 'Wonder Cot', 'Goldrich', 'Aurora', 'Tsunami', 'Bettinka', 'Orange Red' and 'Spring Blush' which were starting flowering first. The flowering of the mentioned cultivars started in the period from March 1 to 5. Early flowering of apricot was a result of two heat waves[1] that have appeared in February 2021 (Figure 1).

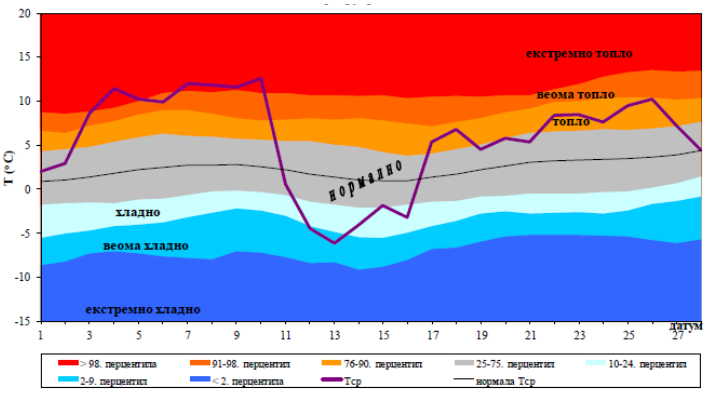


Figure 1. Daily mean temperatures in February 2021 (http://www.hidmet.gov.rs)

On the other hand, there were two cold waves during the period from March 5 to 7, as well as from March 15 to 22, (Graph 2), so flowering started much later in other cultivars. The average air temperature, by the percentile method, in March 2021 was in the normal category in most of Serbia, while in the central and southern parts, it was in cold category, so we had a very "stretched" flowering. Blasse and Hofmann (1993) stated that the air temperature must remain above +4 °C, for the started flowering to be normal. 'Hungary Best' started flowering on March 16, 'NS-4' and 'Farbaly' on March 20, and 'Roxana' and 'Zaklopačka Ruža' on March 24.

Differences in start of flowering among cultivars in 2021 were as much as 23 days. The duration of flowering (average value per one cultivar) was 8 to 16

[1] According to the percentile method, a heat wave is a period during which the maximum daily air temperature in the domain is very warm and extremely warm for five or more days.

days. The total duration of apricot flowering (average value for all cultivars) was 35 days in 2021.

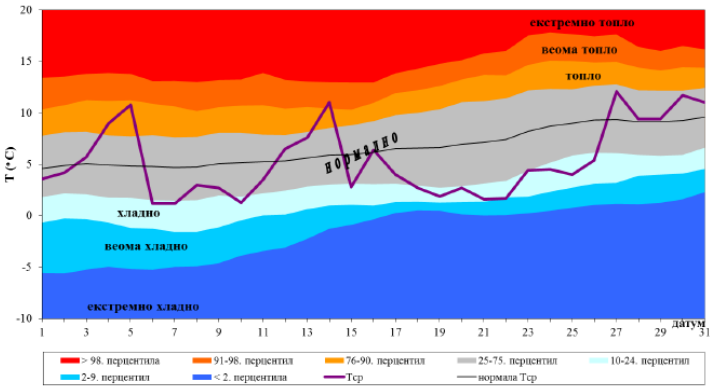


Figure 2. Daily mean temperatures in March 2021 (http://www.hidmet.gov.rs)

Flowering in 2022. Start of flowering of all tested cultivars in 2022 was differed by only 3 days. 'Wonder Cot' and 'Spring Blush' started flowering on March 24; 'Goldrich', 'Aurora', 'Tsunami' and 'Orangered' on March 25; 'Hungary's Best', 'Betinka', 'Roxana' and' Farbaly' on March 26 and 'NS-4' and 'Zaklopačka Ruža' on March 27. The duration of flowering (for one cultivar) was 6 to 9 days in 2022. The total duration of flowering in all examined apricot cultivars in 2022 was 12 days.

Apricot is fruit species which is characterized by early flowering. In addition to the genetic characteristics of the cultivar, the flowering time is influenced by the weather conditions before and during flowering. Milatović (2013) noted that the variation in flowering between years is four to five times higher than the variation between cultivars. This means that flowering time is more influenced by environmental factors (especially temperature) than bygenotype. Apricot flowering begins when mean daily temperatures drop at 10-15°C, and maximum daily temperature reach 15 to 20°C. The average monthly air temperature in the months before the beginning of flowering (January and February) is not necessarily decisive for the beginning of flowering, as in 2020 and 2021. According to the average monthly air temperature, February 2020 was warmer than February 2021, and there were two heat waves in February 2021. But, there was also a part of the February 2021 (6 days) when the mean monthly temperature curve was in cold to very

cold range according to the percentile method, which caused lower mean monthly air temperature.

In 2022, the average monthly temperature in February was almost the same as in February 2021, but the apricot flowering started much later. This is due to the fact that the average air temperature, according to the percentile method, in March 2022, was in cold category in most parts of Serbia.

The difference in the beginning of flowering among early and late flowering cultivars was only 3 days in 2022. In 2020, flowering duration was 6 days, while in 2021 it was 23 days. The average value of the difference between the cultivars with the earliest and the latest flowering time is 7 to 10 days under conditions of Serbia (Milatović, 2013). According to the results of Glišić et al. (2017), the difference was higher in years with earlier flowering, while in years with later apricot flowering this difference was much smaller and amounted 2-3 days. In areas with warmer climates, such as Italy and Spain, these differences in apricot flowering were significantly larger (Della Strada et al., 1989; Rodrigo and Herrero, 2002; Ruiz and Egea, 2008). Contrary, these differences were smaller in countries with a colder climate (Vachůn, 2003; Szalay et al., 2006). The duration of flowering in cultivars ranged from 6 to 9 days in 2022 to 8 to 16 days in 2021. Milatović (2005) notes that the duration of flowering of certain apricot cultivars ranges from 5 to 17 days. Licznar-Małańczuk and Sosna (2005) state thatthe best flowering period for 'Hungary Best' lasted an average of 8 days (3-13 days), over a ten-year period. Our results are consistent with thhese reports. According to the results of Milatović et al. (2000) and Glišić et al. (2017) an earlier begining of flowering usually leads to a longer duration of the flowering period, which was confirmed by the results of our work.

According to the average values of the beginning of flowering of the tested apricot cultivars, 'Goldrich', 'Wonder Cot', 'Aurora' and 'Tsunami' can be classified in the group of very early flowering cultivars. The cultivars 'Spring Blush', 'Betinka' and 'Orange Red' are early-flowering cultivars. 'Hungary Best' was mid-flowering cultivar, while 'NS-4', 'Roxana', 'Farbaly' and 'Zaklopačka Ruža' were late-flowering cultivars according to the time of flowering.

Conclusion

The following conclusions can be drawn from the results presented:

- The average date for beginning of flowering of the tested apricot cultivars was on March 12 in 2020, March 1 in 2021, and March 24 in 2022.

- Duration of flowering in tested cultivars was between 6 and 9 days in 2022, and between 8 and 16 days in 2021.

The difference in the beginning of flowering among the cultivars with the earliest and latest flowering dates was only 3 days in 2022, 6 days in 2020, while this difference was as the highest in 2021, 23 days. The differences in the beginning of flowering among the cultivars were more pronounced in years when flowering began earlier.

Acknowledgement

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'DIVNA' AND 'PETRA' NEW LATE RIPENING PLUM CULTIVARS RELEASED AT FRUIT RESEARCH INSTITUTE, ČAČAK

Nebojša Milošević[1], Ivana Glišić[1], Milena Đorđević[1], Slađana Marić[1], Sanja Radičević[1], Darko Jevremović[1]

Abstract: In this study, the most important characteristics of tree vigour and productivity, as well as pomological traits (fruit morphometric and chemical characteristics) of new late ripening cultivars 'Divna' and 'Petra' released at Fruit Research Institute, Čačak were evaluated and compared to standard cultivar 'Stanley'. Both new cultivars had slightly larger tree and yield in comparison to 'Stanley', but smaller fruit in general. 'Petra' had the best chemical properties of fruit followed by 'Divna'. Both new cultivars could be very interesting for growing in new orchards due to very late ripening time, good fruit quality and high cropping potential and also could be used in further breeding activities.

Keywords: plum, new cultivars, ripening time, fruit properties, yield

Introduction

So far, over 6000 plum cultivars originated from 19 to 40 species and used for fresh market, processing, canning, drying and deep freezing is recognized all over the world (Milošević and Milošević, 2018). Regardless that, there is an intensive work in many breeding programmes worldwide on developing new cultivars which have been realising every year. In European plum (*Prunus domestica* L.), commonly grown in temperate climate zone in Europe including Serbia, the most important breeding objectives are similar in majority of programmes and include: large quality fruits of dark blue colour, yield performance and Sharka (*Plum pox virus*) tolerance/resistance (Milošević and Milošević, 2018; Milošević et al., 2021; Neumüller et al., 2021). Also, certain programmes have their own specific breeding goals.

One of the oldest and the most succesfull plum breeding programme in the Europe has been at the Fruit Research Institute, Čačak, which started in early 1950s of the 20th century, shortly after the founding of the Institute. This programme has been mainly in accordance with demands and needs of producers and includes, among common objectives, also specific such as very

[1]Fruit Research Institute, Čačak, Kralja Petra I/9, 32000 Čačak, Serbia (mnebojsa@ftn.kg.ac.rs)

early and very late ripening time, self-fertility, medium tree size, tolerance to climate change and resistance to the most important funghal diseases (Glišić et al., 2018: Milošević et al., 2021). As a result of this long-term breeding work, so far 18 plum cultivars have been named and released. Some of these cultivars ('Čačanska Lepotica', 'Čačanska Rodna' and 'Čačanska Najbolja') have been well-known and used for production and further breeding worldwide (Jacob, 2002; Hartman and Neümuller, 2006; Milošević et al., 2021).

In 2018, two new late ripening cultivars 'Divna' ('Stanley' × 'Čačanska Rana') and 'Petra' ('Stanley' × 'Opal') were named and released. The main goal of this study was to evaluate and present the most important characteristics of tree vigour, yield, morphometric and chemical characteristics of fruits of these new cultivars in comparison to standard cultivar 'Stanley'.

Materials and methods

Plant material and experimental design. Characteristics of tree, yield and fruit of new plum cultivars 'Divna' and 'Petra' and standard cultivar 'Stanley' were evaluated. The trial was conducted at the Ljubić facility of the Fruit Research Institute, Čačak (43°53'N, 20°20'E, 250 m a.s.l.) at the plum orchard planted in March 2011 using standard one-year-old nursery trees grafted on Myrobalan (*Prunus cerasifera* Ehrh.) seedlings. Trees have been trained as pyramidal crown and grown under standard practices for plum, without irrigation applied. The experiment was designed as a randomized block system in three replicates with 5 trees each (total 15 trees of both new cultivars and standard cultivar).

Vigour and yield characteristics. Trunk circumferences (cm) were measured at 20 cm above the graft union using the digital caliper gauge (Kronen, Germany) and used to calculate the trunk cross-sectional area (TCSA) (cm^2). Yield per tree (Y) (kg) in 2020 and 2021 as well as cumulative yield (CY) in period (2016-2021) was determined using an ACS System Electronic Scale (Zhejiang, China). The yield efficiency (YE) was calculated by dividing values for Y and TCSA.

Morphometric properties of fruits. Twenty-five fruits from both new cultivars and standard of each of two replicates were collected and fruit (FW) and stone weight (SW) (g) were measured using an Ohaus Adventurer technical scale (Parsippany, NJ, USA). Flesh percentage (FP) (%) was calculated as the ratio of the weight of the edible part of the fruit to the total fruit weight. Fruit linear dimensions: hight (H), width (W) and thickness (T) (mm) were measured using a digital caliper Kronen (Kronen GmbH, Kehl am Rhein, Federal Republic of

Germany). Geometric mean diameter of fruit (Dg) was calculated according to formula: $D_g=(LWT)^{1/3}$. Thereafter, sphericity was obtained as the Dg/H ratio.

Chemical properties of fruits. Chemical properties of fruits were examined in the stage of commercial maturity. Soluble solids content (SSC) (%) was assessed by a binocular refractometer (Carl Zeiss, Germany) at 20°C. The total (TS) (%) and invert sugars (IS) (%) content were determined on triplicate samples by the Luff - Schoorl method previously described by Schneider (1979). The sucrose content (SU) was calculated according to the relationship: SU = (TS – IS) × 0.95. The results were expressed in % of fresh weight. The fruit juice pH was assessed by a CyberScan 510 pH-meter (Nijkerk, Netherlands). Total acids (TA) (%) were expressed as malic acids and determined by titration with 0.1 N NaOH up to pH 8.1, using phenolphthalein as an indicator. Once the SSC and TA contents were assessed, the ripening index (RI) was calculated as SSC/TA ratio.

Organoleptic properties of fruits. Organoleptic properties of fruits of the studied cultivars were examined accordance to the guidelines for testing the values for cultivation and use of plum cultivars specified by the Regulations of the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia. Attractiveness (0–6), taste (0–8), aroma (0–4) and consistency (0–2) of fruits were obtained by positive scoring by five panellists. The overall organoleptic score (0–20) is the total of all individual points.

Data analysis. The obtained results were statistically analysed by analysis of variance (ANOVA) using the software package Microsoft Office Excel 2003. The means were separated by LSD test at $p \leq 0.05$.

Results and discussion

Plum tree vigour is dependant on genotype (Nenadović-Mratinić et al., 2007), rootstock, environmental conditions (Blažek and Pištěková, 2009), training system, orchard management and yield (Vitanova et al., 2007). In this work, tree vigiur was presented as TCSA and 'Divna' and 'Petra' had slightly larger tree compared with standard cultivar ('Stanley'). In our previous studies (Milošević et al., 2016; 2019), we found similar tendencies among these cultivars which confirm assumption that both new cultivars could be classified as a moderate vigorous, considering that 'Stanley' is moderate vigour cultivar (Milatović, 2019).

Y was the highest in 'Petra' while in 'Divna' and 'Stanley' was significantly lower (Table 1). On the other hand, CY (2016–2020) was significantly higher in 'Petra' and 'Divna' than in 'Stanley' (Table 1).

Table 1. Tree growth and productivity of new cultivars 'Divna' and 'Petra' and standard cultivar 'Stanley'

	Yield per tree (kg)	Cumulative Yield (kg)	TCSA (cm^2)	Yield efficiency
'Divna'	27.48±0.37 b	122.97±0.74 a	59.49±0.29 ab	0.46±0.00 a
'Petra'	29.66±0.28 a	129.54±0.78 a	63.03±1.47 a	0.47±0.01 a
'Stanley'	27.66±0.26 b	110.69±3.65 b	57.06±1.20 b	0.47±0.01 a

*The different lower-case letters assigned to columns show significant differences for P≤ 0.05 after applying LSD test.

These results are higher then previous obtained by Glišić et al. (2018) and Milošević et al. (2016, 2019) for the same cultivars, probably because of more suitable weather conditions and precipitations over the examined years, as well as differences in orchard management and fertilization. YE was high in all cultivars thanks to large yield and moderate tree vigour, but differences were not significant (Table 1). However, our results were in harmony with perevious reports for different plum cultivars (Glišić et al., 2016; Milošević et al., 2018).

FW is one of the most important quantitative traits which affects yield, fruit quality characteristics and consumers' acceptability (Crisosto et al., 2004). This trait is mostly dependant on genotype, yield and cultural practices in the orchard (Grzyb and Sitarek, 2006). In this work, FW was significantly smaller in both new cultivars compared to 'Stanley' (Table 2).

Table 2. Fruit and stone weight and flesh percentage of new cultivars 'Divna' and 'Petra' and standard cultivar 'Stanley'

	Fruit Weight (g)	Stone Weight (g)	Flash Percentage (%)
'Divna'	27.54±0.14 c	1.28±0.00 c	95.34±0.02 a
'Petra'	29.90±0.15 b	1.36±0.01 b	95.44±0.02 a
'Stanley'	34.58±0.11 a	1.90±0.02 a	94.50±0.03 b

*The different lower-case letters assigned to columns show significant differences for P≤ 0.05 after applying LSD test.

These results were higher than our previous for 'Divna' (Glišić et al., 2018) and 'Petra' (Milošević et al., 2019) and also for 'Stanley' reported by Molnár at al. (2016) and Dimkova et al. (2018). Similar situation was observed with SW, which was significantly smaller in 'Petra' and in particular in 'Divna' regarding to 'Stanley' (Table 2). Thanks to the small stone, FP in 'Divna' and 'Petra' was

similar and significantly larger in comparison to 'Stanley'. Generally, FP is preferable to be as large as possible and both new cultivars meet this criterion.

Fruit dimensions are directly related to the fruit size which is one of the main parameters for fresh market and consumer acceptance of plum (Singh and Singh, 2008). These characteristics are also important for postharvesting operations such transporting, sorting, grading, packaging and storage processes and also in processing operations (Jannatizadeh et al., 2008). In our work, significant differences among the fruit dimensions were found (Table 3). 'Petra' had the largest H, while 'Stanley' had the largest W and T. On the other hand, all these parametrs were the smallest in 'Divna'. Also, the largest Dg was found in 'Stanley' which was expected because two of three dimensions were the highest in this cultivar.

Table 3. Morphometric properties of new cultivars 'Divna' and 'Petra' and standard cultivar 'Stanley'

	Fruit Height (mm)	Fruit Width (mm)	Fruit Thickness (mm)	Geometric Mean Diameter	Sphericity
'Divna'	44.99±0.13 c	32.37±0.08 c	31.05±0.12 c	35.63±0.02 c	0.79±0.00 b
'Petra'	48.30±0.20 a	33.29±0.18 b	32.63±0.03 b	37.43±0.10 b	0.77±0.00 c
'Stanley'	47.12±0.14 b	38.93±0.03 a	33.01±0.07 a	39.29±0.07 a	0.83±0.00 a

*The different lower-case letters assigned to columns show significant differences for $P \leq 0.05$ after applying LSD test.

Results for fruit dimensions in our work was similar to our previous results (Milošević et al., 2016, 2019) and results of Glišić et al. (2018), whilst our results were higher in comparison to results reported by Molnar et al. (2016) and Dimkova et al. (2018). These discrepancies may be associated with different climatic and soil conditions, as well as different orchard management. Overall, according to classification reported by Milatović (2019) both 'Divna' and 'Petra' can be classified as medium sized cultivars. The consumers of plum fruits in the Republic of Serbia and also in most of Europe, prefers elongated fruits compared to spherical (Milošević and Milošević, 2018). There were significant differences among all cultivars concerning this trait and obtained values showed that all of them had elongated fruits which is particularly relevant to 'Petra'.

Concerning chemical properties in this study, significant differences were observed among cultivars 'Divna', 'Petra' and 'Stanley' (Table 4). The highest SSC, TS and SU content was found in 'Petra' and the smallest in 'Divna'.

Amount of IS was similar in 'Divna' and 'Stanley' and significantly higher in comparison to 'Petra'.

Table 4. Chemical properties of new cultivars 'Divna' and 'Petra' and standard cultivar 'Stanley'

	Soluble Solids Content (%)	Total Sugars (%)	Invert Sugars (%)	Sucrose (%)
'Divna'	19.95±0.03 b	12.55±0.02 c	7.12±0.02 a	5.16±0.00 b
'Petra'	21.72±0.08 a	12.92±0.03 a	6.64±0.06 b	5.97±0.09 a
'Stanley'	19.10±0.06 c	12.80±0.02 b	7.24±0.03 a	5.28±0.03 b

*The different lower-case letters assigned to columns show significant differences for P≤ 0.05 after applying LSD test.

'Divna' had the highest amount of TA, and smallest pH value, which means that this cultivar had the most acidic fruit (Table 5). This high content of TA caused the smallest ripening index in 'Divna'. In contrary, the highest SSC and the smallest amount of TA affected the highest RI in 'Petra'.

Table 5. Other chemical properties of new cultivars 'Divna' and 'Petra' and standard cultivar 'Stanley'

	Total Acids (%)	pH	Ripening Index
'Divna'	1.01±0.03 a	3.80±0.02 b	19.81±0.61 c
'Petra'	0.82±0.01 b	3.91±0.01 a	26.58±0.16 a
'Stanley'	0.85±0.01 b	3.90±0.01 a	22.41±0.21 b

*The different lower-case letters assigned to columns show significant differences for P≤ 0.05 after applying LSD test.

In this study, 'Petra' and 'Divna' showed similar tendencies in terms of SSC, TS, IS and SU, as well as TA to our previous results (Milošević et al., 2019) and results of Glišić et al. (2018) in similar conditions which confirms theory that genotype had very high impact on these properties (Usenik et al., 2014). On the other hand, in this work, the content of TS and IS was lower, while the content of TA was higher compared to results obtained in our previous work regarding the same cultivars but grown in different conditions (Milošević et al., 2016), which showed that climate, soil and orchard management also influenced these characteristics. Overall, plums SSC ≥ 12.0% had ~75% consumer acceptance, regardless of TA (Crisosto et al., 2004). Therewith, various organic acids and their relative contents differ in the level they have an

effect on sugars (Colarič et al., 2005). In this regard, the assumption is that 'Divna' and 'Petra' could satisfy main consumer requirements.

Table 6. Chemical properties of new cultivars 'Divna' and 'Petra' and standard cultivar 'Stanley'

	Attractiveness (0–6)	Flavour (0–8)	Aroma (0–4)	Consistency (0–2)	Total (0–20)
'Divna'	5.00	6.00	1.00	3.00	15.00
'Petra'	5.00	7.00	2.00	3.00	17.00
'Stanley'	5.00	6.00	1.00	3.00	15.00

The subjective perception of the fruit quality by the consumers is very important in the final estimation and acceptance of new cultivar (Crisosto et al., 2007). New plum cultivars 'Divna' and 'Petra' examined in this work showed similarities among each other and standard cultivar 'Stanley' with small differences in 'Petra' (Table 6). Only distinctions were observed in flavour and teste where 'Petra' was slightly better assessed than other two cultivars. On some way, it was expected, because 'Stanley' is one of perents of both other cultivars. In total, both new cultivars were highly rated for all organoleptic traits which indicates that they could be well accepted from consumers.

Conclusion

Evaluation of the most important tree growth and yield characteristics, morphometric, chemical and organoleptic properties of new cultivars 'Divna' and 'Petra' released at Fruit Research Institute, Čačak and standard cultivar 'Stanley' indicated that both cultivars were characterized by very good productive and fruit quality traits and could be very interesting for growing in new orchards in the Republic of Serbia. Very significant preference of these cultivars is their very late ripening time, especially if it is known that there is a small number of cultivars that ripens at a similar or the same time. These both cultivars could be interesting as a perents in further breeding programme at the Fruit Research Institute, Čačak or other plum breeding programmes.

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POMOLOGICAL PROPERTIES OF SELECTED WALNUT GENOTYPES FROM THE NATURAL POPULATION

Ivana Jasnić[1], Slađana Janković[1], Dragan Janković[1], Dragan Milatović[2], Dragan Grčak[1], Milosav Grčak[1]

Abstract: The pomological characteristics of selected walnut genotypes from the natural population in the municipality of Leposavić were examined. The study included 33 walnut genotypes from the natural population in the valley of the river Ibar. Due to the permanent generative reproduction, the walnut population is very heterogeneous. The main goal of the research is to find and describe promising genotypes that can be used in breeding work or for establishing new plantations.Obtained results showed that inthe natural population of walnuts, the genotypes of poorer traits predominate, but several genotypes of exceptional characteristics weresingled out, which are interesting for further research.

Keywords: walnut, natural population, genotype, pomological characteristics.

Introduction

In Serbia, there are significant natural populations of walnut, most often in river valleys, which indicates that this fruit species is suitable for habitats with fertile and sufficiently moist soil. As a result of generative propagation and pronounced cross-fertilization, natural walnut populations are characterized by a large number of different genotypes, which potentially represent a wide base for selection. A large number of domestic walnut cultivars were created by selection from natural populations. Numerous authors worked on individual positive selection of walnutgenotypesfrom natural populations in Serbia and a large number of promising selections with a number of favorable traitswas selected and described (Mitrović et al., 2007;Cerović et al., 2010; Cerović et al., 2014).In addition to Serbia, walnut selection is also being done in other countries such as Turkey, Iran, Italy and others (Akca et al., 2015; Poggetti et al., 2017; Khadivi et al., 2019).

[1] University of Priština, Faculty of Agriculture, Kopaonička bb, Lešak, Serbia (ivana.jasnic@pr.ac.rs)
[2]University of Belgrade, Faculty of Agriculture, Nemanjina6, Belgrade, Serbia

In the valley of the river Ibar there is a large number of local, insufficiently researched natural populations of walnut. After Đurđević (1968), in Kosovo and Metohija no studies of the walnut population have been carried out until today. In the area of Leposavić municipality, walnut has been traditionally grown on homesteads or grows spontaneously in free conditions for decades. In such a long period of time, a large number of new genotypes were created that can be expected to possess positive characteristics.

Materials and methods

The research included 33 walnut genotypes from the natural populationin the valley of the river Ibar, in the municipality Leposavić. Selected trees are marked with numbers. At the stage of full fruit maturity, in the fall of 2021, fruits were collectedby random sampling for each genotype, from different parts of the tree. Four replications were performed and 20 fruits were harvested in each replication.Fruits harvested from one tree constituted one sample.

After drying the fruits, measurements were made and the following characteristics were monitored: nut weight, kernel weight, kernel percentage and ease ofkernel removal from the nut. The average value was obtained as the mean value of 10 tested fruits from the sample. The evaluation of the ease ofkernel removal from the nutwas carried out according to the IPGRI descriptor for walnuts (1994).

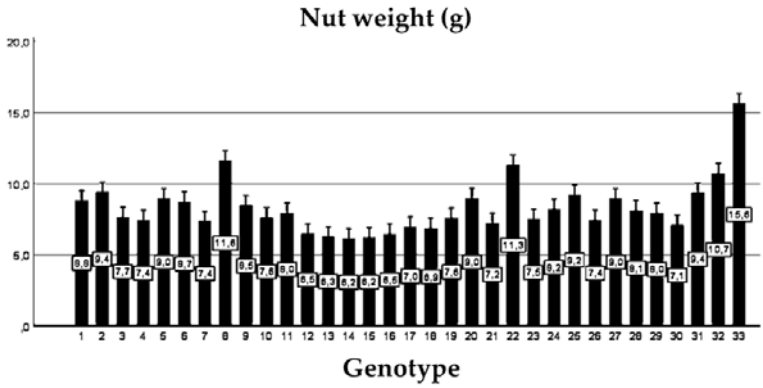
All measurements were performed with precise analytical scalesKern EHA 3000-0 (Kern& Sohn GmbH,Germany). The nuts were opened by careful blows with a hammer. Analysis of the obtained data was performed by applying the Fisher model of variance analysis. In cases of statistical significance of the F indicator in the factor, the significance of the differences between the mean values of the examined parameters was performed using the Tukey test (TUKEY HSD test) for threshold significance $p < 0.05$.

Results and discussion

Differences in genotype influenced differences in nut quality within theexaminedpopulation. Genotypic differences were highly significant ($p = 0.000$) in all examined characteristics. Each individual tree represents one genotype, considering the generative reproduction of walnuts as the only type of propagation under the examinedconditions.

Nut weight

The total average weight of the nut in the examined walnut genotypes was 8.3 g. Genotypes 14 and 15 (6.2 g) had the lowest nut weight, and genotype 33 (15.6 g) had the highest nut weight. In addition to genotype 33, genotype 8 with a nut weight of 11.6 g, as well as genotype 22 with a nut weight of 11.3 g, had a significantly higher nut weight than the other genotypes (Graph 1).



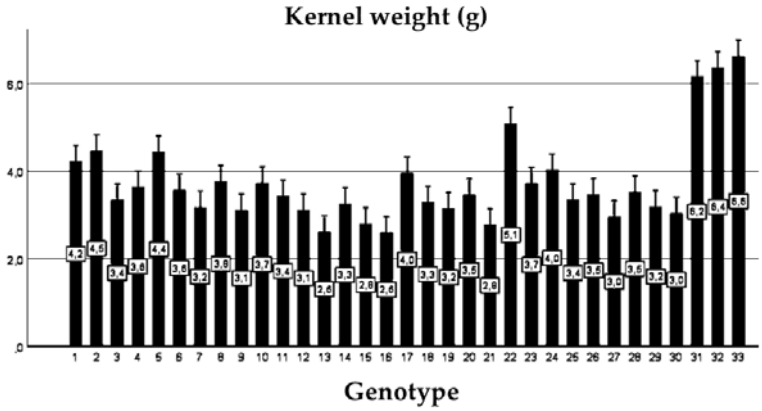
Graph 1. Average valuesof nut weight of the examined genotypes

According to Cerović et al (2010), 7.2% of the examined population had a nut weight over 14 g, while our results showed that only one genotype (3.0% of the examined natural population) had a nut weight over 14 g.

Kernel weight

The total average weight of the kernel in the tested walnut genotypes was 3.7 g.Genotypes 13 and 16 (2.6 g) had the lowest kernel weight, and genotype 33 (6.6 g) had the highest. In addition to genotype 33, genotype 31 with a kernel weight of 6.2 g, as well as genotype 32 with a kernel weight of 6.4 g, had a significantly higher kernel weight than the other genotypes (Graph 2).

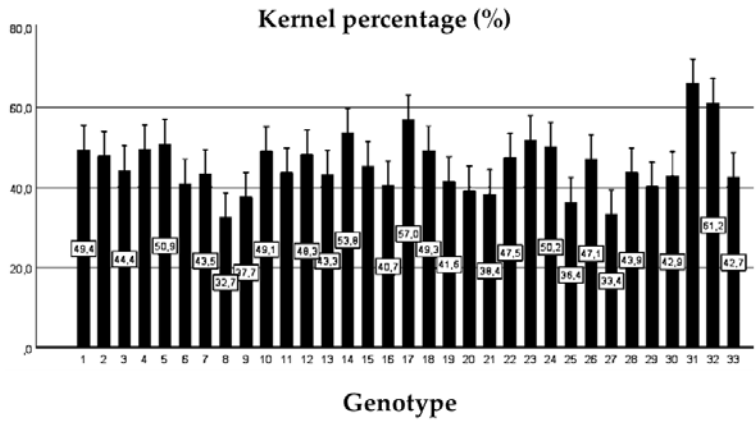
Jaćimović and Božović (2017) found that among the five examined varieties and selections of walnuts, the highest kernel weight was recorded in the'Šampion'variety (7 g), and the smallest in the 'Elit' variety (4.2 g).



Graph 2. Average values of kernel weight of the examined genotypes

Kernel percentage

The total average percentage of kernels in the examined walnut genotypes was 45.8%. The lowestkernel percentagewas 32.7% (genotype 8), and the highest was 66% (genotype 31). In addition to genotype 31, genotype 32 (61.2%) and genotype 17 (57.0%) had a significantly higher kernel percentage than other genotypes (Graph 3).



Graph 3. Average values of the kernel percentage of the examined genotypes

The kernel percentage of genotype 31 surpasses the kernel percentage of genotypes from natural populations studied by Cerović et al. (2010) and Paunović and Miletić (2013).

Ease of kernel removal from the nut

This characteristic means that the internal partitions are not tightly fused with the shell, that the kernel is easily removed and remains whole, in one piece(Figure 1).From the examined samples, the kernel was difficult to separate in 12 genotypes, medium easy in 10, and easy in 11 genotypes. Nuts of genotypes 4, 5, 8, 10, 11, 14, 17, 28, 31, 32 and 33 had kernels that were easily removed and remained whole.

Figure 1. The kernel remains whole after opening the nut

Conclusion

Generative propagation of walnut as the only type of reproduction resulted in a rich gene pool, so practically this entire area is a selection field that provides a basis for further research.

The average nut weight of investigated walnut genotypes varied from 6.2 g to 15.6 g. The kernel weight ranged from 2.6 g to 6.6 g. The highest kernel percentage was 66% and the lowest was 32.7%. More than 87% of the examined nuts had a kernel percentage greater than 40%.

Among the selected genotypes, those with lower fruit quality predominate, but it should be noted that several genotypes with good pomological characteristics were singled out.Genotypes 14, 17, 22, 31, 32 and 33 stand out in terms of favorable pomological traits. Genotype 31 proved superior to other genotypes, and is therefore interesting for further research. The most significant features of this genotype are high kernel percentage and ease of kernel removal from the nut.

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PRODUCTION AND TECHNOLOGICAL CHARACTERISTICS OF PROSPECTIVE VINE HYBRIDS IN THE NIS WINE-GROWING REGION

Mlađan Garić[1], Ivana Radojević[2], Dragan Nikolić[3], Vera Rakonjac[3], Aleksandar Petrović[3], Zorica Ranković-Vasić[3]

Abstract: This paper presents the results on the study of promising vine hybrids in the Nis wine-growing region. The following promising vine hybrids were obtained from three different crossing combinations: NI 11-92 (Prokupac x Gamay Noir), NI 8-92 (Smederevka x Traminer Savagnin rose), and NI 2-92 (Smederevka x Riesling Rhine). The hybrids were created in the "Centre for Viticulture and Winemaking" in Niš.

The tested hybrids differed in their cropping potential, grape yield and quality, chemical composition and sensory evaluation of the wine.

Key words: phenology, hybridization, yield, grape quality, wine quality.

Introduction

The aim of vine breeding is to obtain new high-yielding and high-quality varieties. This goal can be achieved most quickly by crossing between different species. Various methods of vine breeding have created many new varieties that have helped to increase yields many times over, maintain or improve the quality of grapes and to increase the resistance of many varieties to adverse environmental factors (Singh and Murthy, 1993; Nikolić, 2018). The creation of new vine varieties in our country started in the 1960s. A certain number of newly created varieties have been studied and described in detail by many authors (Avramov, 1991; Burić, 1995; Tarailo *et al.*,1997; Garić *et al.*, 1998; Cindrić *et al.*, 2000; Korać *et al.*, 2002; Nikolić, 2018; Žunić and Garić 2017) and others. At the experimental estate of the "Centre for Viticulture and Winemaking" in Nis, work has been going on for several years on the creation

[1]University of Kragujevac, Faculty of Agronomy Čačak, Cara Dušana34, Čačak, Serbia (mladjangaric1963@gmail.com)
[2]Ministry of Agriculture, Forestry and Water Management, Sector of Agricultural Inspection, Department for Wine, Brandy, Alcoholic and Non Alcoholic Beverages, Omladinskih brigada 1,11070 Novi Beograd, Serbia
[3] University of Belgrade, Faculty of Agriculture, Nemanjina 6, Belgrade, Serbia

of new vine varieties by hybridization, with the aim of combining the desirable characteristics of selected parents in the genotype of one of their offspring. A large number of hybrids have been obtained from numerous crossing combinations, which are interesting for recognition as a new variety or for further breeding. This paper presents the results of testing the biological and productive characteristics of promising vine hybrids from three combinations of crosses between varieties.

Materials and methods

The tests were carried out in the vineyard of varieties collection of the "Centre for Viticulture and Winemaking" in Nis. The object belongs to the Nis wine-growing region. The altitude of the object is 210 m, the slope of the terrain is gentle and the exposure is north. The vineyard was planted in 1995. year on an area of 2 ha, with over 200 vine genotypes. Newly created cross-varietal vine hybrids were used as test material, resulting from three different crossing combinations: NI 11-92 (Prokupac x Gamay Noir), NI 8- 92 (Smederevka x Traminer/Savagnin rose), and NI 2-92 (Smederevka x Riesling Rhine). All the newly created wine hybrids were planted at a distance of 3.0 x 1.2 m. The rootstock on which they are grafted is Berlandieri x Riparia Kober 5 BB. The training system is the "Karlovac cultivation form" with a tree height of 80 cm, using mixed pruning . The support is a trellis consisting of a wooden stake, a concrete pillar and wire serves as support and ensures correct arrangement of the shoots, leaves and grapes in the space. When pruning, a condir with 2 meshes and two curves with 10 meshes each were left on the vine wood. In this way, all vine woods were loaded with the same number of buds, eliminating the influence of different vine loads on the yield and quality of the grapes. The tests were carried out in the period 2011-2013 and were divided into two parts: the field part of the experiment and the laboratory chemical analysis of grapes and wine. The field part of the experiment included 10 vine wood cuttings for each treatment. The experiment was conducted using the random selection method, with ten repetitions (10 vine woods within each variant, with each vine cutting serving as a separate experimental unit. The laboratory tests were carried out in the laboratories of the "Centre for Viticulture and Winemaking" in Nis, the Agricultural Extension Service in Nis and the Faculty of Agriculture in Belgrade. The research included the following indicators: phenological observations, reproductive potential, yield and mechanical composition of the

grapes, quality of the grapes, chemical composition of the wine and wine regulatory assessment.

Results and discussion

The timing of certain phenophases in the annual development cycle of the examined hybrids could be seen read the data shown in Table 1. The hybrid NI 11-92 had the earliest activation of the bud burst (12 April), the beginning of flowering (30 May) and the ripening time (28 September). The latest activation of buds (17 April) and ripening time (30 September) were found in the hybrid NI 8-92 , while the hybrids NI 8-92 and NI 2-92 had the same start of flowering (31 May).

Table 1. Mean values of the phenological phases of the tested hybrids (average 2011-2013. years)

Characteristic	Hybrid		
	NI 11-92	NI 8-92	NI 2-92
Activation of bud burst	12.04.	17.04.	13.04.
Beginning of flowering	30.05.	31.05.	31.05.
End of flowering	12.06.	16.06.	12.06.
Development of Berries	19.06.	21.06.	18.06.
Maturation	30.07.	30.07.	04.08.
Time of ripening	28.09.	30.09.	29.09.
Number of days	169	165	169

Grape yield and quality

From the data shown in Table 2, it can be seen that the highest grape yield per vine (4.55 kg), number of bunches per vine (24.83) and width of the bunch (9.47 cm) were recorded for the hybrid NI 8-92, while the highest average weight of grapes was for the hybrid NI 2-92 (183.75 g).

Table. 2. Mean values and indicators of yield variability, grape quality and characteristics of the examined hybrids (average 2011-2013)

Characteristic	Hybrid								
	NI -92			NI 8-92			NI 2-92		
	$\bar{X}$	S	Cv(%)	$\bar{X}$	S	Cv(%)	$\bar{X}$	S	Cv(%)
Grape yield per vine wood (kg)	3.52	0.67	18.93	4.55	0.99	21.68	3.65	0.55	15.02
Number of bunches per vine wood	20.50	3.06	14.93	**24.83**	3.91	15.76	20.10	2.35	11.71
Bunch mass (g)	177.89	39.95	22.46	181.95	45.40	24.95	**183.75**	33.41	18.18
Bunch length (cm)	**16.27**	2.77	17.00	14.00	1.84	13.13	13.93	2.52	18.07
Bunch width (cm)	8.27	1.76	21.29	9.47	1.66	17.49	7.63	1.75	22.95
Number of berries per bunch cluster	**140.30**	44.41	31.65	89.00	34.01	38.21	83.80	17.40	20.76
Mass of gooseberries (g)	5.96	1.58	26.58	2.69	0.82	30.30	5.01	1.45	28.98
The sugar content in the must (%)	**24.03**	1.68	6.98	21.52	1.37	6.36	21.65	1.22	5.64
Content of total acids (g/l)	7.83	0.81	1.34	7.80	1.20	15.34	**8.34**	0.90	10.83
Alcohol content in the wine (% Vol.)	13.76			13.23			12.92		
Sensory evaluation of wine	63			66			64		

The hybrid NI 11-92 had the highest average values for bunch length (16.27), number of berries per bunch (140.30) and gooseberry mass (5.96 g). The hybrid NI 11-92 had the lowest grape yield per vine wood (3.52 kg) and bunch weight (177.89 g). The lowest number of bunches per vine wood (20.10), bunch length (13.93 cm), bunch width (7.63 cm) and number of berries in a bunch (83.80) were obtained from the hybrid NI 2-92, while the hybrid NI 8-92 had the lowest mass of gooseberries (2.69 g). The greatest variation in grape yield per bunch, number of bunches per bunch, bunch weight, number of berries per bunch and gooseberry weight was found in the hybrid NI 8-92 (Cv = 21.68%; Cv = 15.76%; Cv = 24.95 %; Cv =38.21%; Cv=30.30%), and bunch length and bunch width in hybrid NI 2-92 (Cv = 18.07%; Cv =22.95%). The highest content of sugar in the must was found in hybrid NI 11-92 (24.03%), and the highest content of total acids in the must was found in hybrid NI 2-92 (8.34 g/L). The sugar content in the must of the hybrid NI 8-92 was 21.52%, and in hybrid NI 2-92 it was (21.65%). The content of total acids in the must of the hybrid NI 11-92 was 7.83 g/L and in the hybrid NI 8-92 (7.80 g/l). The greatest variation in sugar content in the must was found in the hybrid NI 2-92 (Cv=6.98%) and the content

of total acids in the must in the hybrid NI 8-92 (Cv=15.34%). The alcohol values in the wine of the tested hybrids ranged from 12.92 % Vol. in the hybrid NI 2-92 to 13.76 % Vol. in the hybrid NI 11-92. The sensory evaluation of the wines of the examined hybrids ranged from 63 to 66 points.

Table 3. Statistical significance of the influence of the year on the yield and grapes characteristics of the examined hybrids

Year		Grape yield per vine wood		Number of bunches per vine wood		Bunch length	
		Environment differences	*by value*	Environment differences	*by value*	Environment differences	*by value*
2011	2012	-0.11^{nz}	0.80	-1.93*	0.03	0.07^{nz}	0.99
	2013	-0.69**	0.00	-2.90**	0.00	-1.77**	0.008
2012	2011	0.11^{nz}	0.80	1.93*	0.03	-0.07^{nz}	0.99
	2013	-0.57**	0.006	-0.97^{nz}	0.41	-1.83**	0.005
2013	2011	0.69**	0.00	2.90**	0.00	1.77**	0.008
	2012	0.57**	0.006	0.97^{nz}	0.41	1.83**	0.005

nz for p >0.05*; for p < 0,05; ** for p<0,01.

Table 3 shows that the yield of grapes per vine wood was statistically significantly higher in 2013 than in 2011 and 2012. The yield of grapes per vine wood was not significantly different between 2011 and 2012. The number of bunches per bush in 2013 was significantly higher than in 2011, and was not different from the number of bunches per bush in 2012. In 2012, the number of bunches per bush was significantly higher than in 2011. The length of the bunch in 2013 was statistically particularly higher than in 2011 and 2012. It was not different significantly between 2011 and 2012.

Table 4. Statistical significance of the influence of genotype and year on the content of sugar and total acids of the must in the examined hybrids

Sources of variation	Sugar content in the must		Content of total acids in the must	
	ANOVA			
	F-value	*by value*	*F-value*	*by value*
Hybrid	34.90**	0.00	3.03^{nz}	0.05
Year	3.53*	0.03	0.21^{nz}	0.81
Hybrid x year	4.00**	0.005	3.60*	0.01

nz za p>0.05;* za p<0.05;** za p<0.01

The results of the analysis of variance in Table 4 showed that very significant differences were found for the content of sugar in the must between

the studied hybrids, while the differences in the content of total acids in the must were not significant. The influence of the year was significant for the sugar content in the must, but it was not significant for the content of total acids in the must ($p>0.05$). The joint influence of genotype and year was very significant for the content of sugar in the must and significant for the content of total acids in the must.

Conclusion

Based on the results of the examination of promising vine hybrids in the Nis wine-growing region, the following conclusions were drawn:

Hybrid NI 11-92 had the earliest budburst activation (12 April), easliest flowering start (30 May) and earliest ripening time (28 September). The latest activation of budbursts (17 April) and ripening time (30 September) were recorded in the hybrid NI 8-92 , while the hybrids NI 8-92 and NI 2-92 had the same start of flowering (31 May).

The highest yield of grapes per vine wood (4.55 kg), the number of bunches per vine wood (24.83) and the width of the bunch (9.47 cm) was recorded for the hybrid NI 8-92, while the highest average weight of bunches was in hybrid NI 2-92 (183.75 g). The yield of grapes per vine wood in 2013 was statistically significantly higher than in 2011 and 2012. The yield of grapes per vine wood was not significantly different between 2011 and 2012. The highest sugar content in the must was recorded in the NI 11-92 hybrid (24.03%), and the total acid content in the NI 2-92 hybrid (8.34 g/l). The content of sugar in the must of the hybrid NI 8-92 was 21.52%, and of the hybrid NI 2-92 it was (21.65%). The greatest variation in sugar content was found in the hybrid NI 2-92 (Cv=6.98%), and in total acid content in the hybrid NI 8-92 (Cv=15.34%). The alcohol values in the wine of the tested hybrids ranged from 12.92 % Vol. in the hybrid NI 2-92 to 13.76 % Vol. in the hybrid NI 11-92. The sensory evaluation of the wines of the examined hybrids ranged from 63 to 66 points. The results of the analysis of variance showed that very significant differences in the sugar content of the must were found between the tested hybrids, and that the differences in the content of total acids of the must were not significant. Under the conditions of the Nis wine-growing region and similar agro-ecological conditions, examined vine hybrids can be grown successfully

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COMPARATIVE STUDY OF PRODUCTIVITY AND FRUIT QUALITY OF STRAWBERRY CULTIVAR 'SENGA SENGANA' GROWN IN THE OPEN FIELD AND PLASTIC-GREENHOUSE

Jelena Tomić [1], Boris Rilak, Marijana Pešaković, Žaklina Karaklajić Stajić, Svetlana M. Paunović

Abstract: To identify and compare the differences in productivity and fruit quality of strawberry cultivar 'Senga Sengana' grown in open field and protected area fruits were harvested in 2021 in the region of Western Serbia. The yield, physical (fruit weight, dimensions, firmness), chemical (total soluble solids (TSS), total (TS) and invert sugars (IS), sucrose (SUC), titrable acids (TA), pH, sweetness index (TS/TA)) and nutritive traits (vitamin C, total phenols, total anthocyanins and antioxidative activity) of the fruits were analyzed. Significantly higher yield per area unit, fruit weight and length, TSS, sugars, organic acids, sweetness index and nutritive qulity were recorded in the fruits from protected area. Growing strawberries in a protected area, in addition to ensuring a safe yield, also has a positive effect on both the sensory and nutritional composition of the fruit.

Keywords: strawberry, yield, physical traits, chemical composition

Introduction

Due to the efforts of growers and the adaptability of Fragaria ananassa plants, strawberry cultivation has spread almost worldwide and world strawberry production in 2020 was nearly 7 million tons (Fecka et al., 2022). According to FAOSTAT, the annual strawberry production in Serbia ranges from 20.000 to 30.000 t, and the area under plantations is about 7.000 ha. The most common growing technology is in the open field on beds covered with black foils and drip irrigation systems. The average yield per ha of strawberry in Serbia is low compared to countries with intensive strawberry production. Low commercial strawberry yields are a consequence of late spring frosts during flowering which caused damage to the flowers and rainy periods during the ripening which promotes the spread of gray mold. In addition, strawberry plantations are established with plants originating from commercial orchards or

[1]Fruit Research Institute, Kralja Petra I 9, Čačak, Serbia (jtomic@institut-cacak.org)

with insufficient application of agro-technical practices, in which yields are low and fruit quality varies from year to year. One of the ways to overcome the mentioned problems is to grow strawberries in protected conditions. However, strawberry plantations in tunnels, plastic-greenhouses or greenhouses in Serbia is less represented (5-10% of total area under strawberry plantations) compare to open field, due to the high costs of establishing such growing systems.

The most common cultivars in strawberry plantations in Serbia are 'Clery', 'Joly', 'Alba', 'Asia', 'Arosa' and 'Roxana'. The largest share in production plantations is occupied by early ripening cultivars 'Clery' and 'Alba', which reach the highest prices on the market. The abovementioned cultivars are economically important, while the ever-bearing cultivars and cultivars intended for processing are grown on a smaller scale. 'Senga Sengana' strawberry has been a leading industrial cultivar for many years (Fecka et al., 2022), both in the world and in Serbia.

Cultivar 'Senga Sengana' was originating from Germany (Sengana GmbH., Wulfsdorf, Hamburg) and despite being created back in 1954, it is still represented in strawberry plantations thanks to its adaptability to drought, moderate susceptibility to frost and high adaptability to extensive cultivation. In the conditions of Serbia, this cultivar ripens late, during the third ten-day period of May. The plant is very strong and vital and produces a moderate number of runnings. It is resistant to many fungal diseases (*Sphaerotheca macularis*, *Phytophtora fragariae*, *Verticillium albo-atrum*, *Verticillium dahlia*) and moderately susceptible to gray mold (*Botrytis cinerea*). The fruit of cultivar 'Senga Sengana' is medium, fringed-conical shape, dark red color. The flesh of the fruit is juicy, sweet-sour taste and aromatic. Fruits are suitable for freezing and processing, but it is also used fresh. However, it is important to note that in the last few years 'Senga Sengana' has become less represented in strawberry plantations in Serbia.

Strawberry fruits produced in Serbia are mostly used fresh for marketing on the domestic and foreign markets, while a smaller part goes into further processing. Strawberry exports are dominated by primary products (fresh and frozen fruit) (Zarić et al., 2015). Fresh strawberries are mainly oriented towards the Russian market, while frozen ones are exported to EU countries and Russia. Bearing in mind that the quality of strawberries produced in Serbia competes both on the domestic and foreign markets (Zarić et al., 2015), as well as the fact that the cultivar 'Senga Sengana' is easy to grow on the one hand and suitable for processing and freezing on the other hand, the aim of this study was to compare productivity and fruit quality of this cultivars in the open field and

protected areas. The obtained results will indicate to the producers the most suitable growing system for strawberry cultivar 'Senga Sengana' for obtaining high yields of quality fruits, which at the same time ensures the economy of production.

Materials and methods

Study area and experimental layout

The experiment was carried out at Milićevo selo, Požega (43°20′07″ N latitude, 20° 05′19″ E longitude, 345 m altitude), in the Zlatibor District. Strawberry plantation at the open field was established in Avgust 2021 in one rows on beds covered with black polyethylene foil. Strawberry plantation at the plastic-greenhouse (protected area) was establish during September 2021 on one beds covered with black agrotextil. Both experimental areas involved economically significant strawberry cultivar 'Senga Sengana' (*Fragaria annanasa* L.), 'frigo' runnings, A category. Plant spacing in both growing systems was 18 cm, and spacing between beds was 50 cm in open field and 40 cm in plastic-greenhouse. In addition to standard cultivation practices, the plants were regularly irrigated according to soil humidity. The fertilizers were applied through fertigation according to the phenological stage of the plant.

The layout of the experiment was a completely randomized design, with the effect of growing system analyzed: open filed and protected area (plastic greenhouse). The experimental area in both growing systems included 100 plants (25 plants in 4 repetitions). Fruit samples for determination of physical and chemical properties were taken at the commercial maturity stage, during the three harvests (beginning, middle and end of harvest), and an average sample of aproximatelly 1 kg per repetition was made.

In both experimental plantations, soil sampling for chemical analysis was performed before planting, and results are shown in Table 1.

Table 1. Chemical soil composition in experimental plantation

Growing system	Humus (%)	N_{TOT} (%)	P_2O_5 (mg 100 g^{-1} a.d.s)	K_2O (mg 100 g^{-1} a.d.s)	$CaCO_3$ (%)	pH (KCl)
Protected area	3.01	0.16	17.69	19.08	0.00	4.80
Open field	3.86	0.19	6.28	22.43	0.69	5.61

Productivity of strawberry

Yield per plant was determined by measurement of weight of fruits in each harvests and summing up the yield from all harvests, while the yield per unit area determined by calculation (multiplying the number of plants per unit area and yield per plant).

Physical properties of strawberry fruit

For physical fruit properties, 25 fruits in four replicates (n = 100) were used. Within physical properties of fruits, weight, dimensions (length and width) and shape index were performed by usual morphometric methods. Fruit weight was determined using a technical balance (Mettler Toledo, Switzerland) with an accuracy of ±0.01 g, and the data were expressed in g per fruit. Fruit dimensions were measured by a digital caliper (Carl Roth, Germany) with an accuracy of ± 0.05 mm. The value of the fruit shape index is obtained by calculation, establishing a relationship between fruit length and width. Fruit firmness is determined by using a penetrometer and values were expressed in N.

The fruit samples on which the physical properties were determined further used for chemical analyses.

Chemical properties of strawberry fruit

Fruit samples were routinely prepared for chemical analysis. Total soluble solids (TSS) expressed as °Brix were measured with a digital refractometer (Pocket PAL-1, Atago, Japan). Titratable acidity (TA) was measured according to AOAC method 942.15 (1995) and expressed as % citric acid. The content of total sugars (TS), invert sugars (IS) and sucrose (SUC) were determined volumetrically, using the Luff-Schoorl method (Egan et al., 1981). pH value was measured with a pH-meter. The sweetness index was calculated as the total sugars and titratable acidity ratio (TS/TA).

Total phenols content (TPC) was determined using a modified Folin-Ciocalteau method (Singleton et al., 1999; Liu et al., 2002). The results were expressed as mg of gallic acid equivalents per 100 g fresh weight of the sample (mg GA eq 100^{-1} g FW). Determination of the anthocyanins content in the fruit was performed using the pH differential method (Torre and Barritt, 1977; Liu et al., 2002). The results were expressed as the mg equivalent of cyanidin-3-glycoside per 100 g of fresh weight (mg C3G eq 100 g^{-1} FW). Content of vitamine C was detected by iodometric titration (Suntornsuk et al., 2002), and results were showed as mg per 100 g of fresh weight (mg 100 g^{-1} FW) Antioxidant capacity was determined using the DPPH method reported by Brand-Williams et al. (1995)

with modifications (Sánchez-Moreno et al., 1998). Trolox was used as an antioxidant standard. The results were expressed as the ascorbic acid equivalent per 100 g of fresh weight (mg AA eq 100^{-1} g FW).

Results and discussion

Fruit quality can be defined by fruit weight and soluble solids content, but the yield is considered the most important parameter of production efficiency. These are the main factors contributing to the direct effect on marketability (Milošević et al., 2012). The yield per plant in our study (Table 2) was not significantly different between studied growing systems (protected area and open field). However, yield per unit area was significantly higher in plastic-greenhouse compared to open field.

Table 2. Productivity of strawberry cultivar 'Senga Sengana'

Growing system	Yield per plant (g)	Yield per area (kg/m^2)
Protected area	419.0±20.0 a	2.1±0.1 a
Open field	423.2±24.5 a	2.0±0.2 b

The different lower-case letters in the columns indicate statistically significant differences among cultivars at P≤0.05 (Duncan's test).

Strawberry cultivars have fruits of variable size, which depends on genotype, environmental factors, cultivation system, age of plantations and ripening time. Strawberry cultivar 'Senga Sengana' in this study differed in terms of weight and length of fruit, indicating the significant influence of growing system on these traits (Table 3). Fruits of cultivar 'Senga Sengana' from protected area was characterized by the significantly higher weight and length (11.9 g and 31.5 mm) related to the fruits from open field (9.9 g and 26.7 mm).

Table 3. Physical fruit traits of strawberry cultivar 'Senga Sengana'

Growing system	Weight (g)	Length (mm)	Width (mm)	Shape index	Firmness (N)
Protected area	11.9±0.9 a	31.5±1.0 a	30.1±0.9 a	1.0±0.1 a	9.3±0.1 a
Open field	9.9±1.7 b	26.7±1.1 b	27.1±1.2 a	1.1±0.1 a	9.5±0.2 a

The different lower-case letters in the columns indicate statistically significant differences among cultivars at P≤0.05 (Duncan's test).

According to Jouquand (2008) strawberry genotypes assessed as 'not sweet' also had low soluble solids content and this parameter is generally a good indicator for the acceptability of consumers. The total soluble solids in our samples was higher in strawberries grown at the protected area (9.30%) compared to the open field condition (7.10%) (Table 4).

The dominant participation in the structure of total sugars in strawberry fruits had invert sugars (glucose and fructose), while sucrose was detected at low concentrations. The sugar content in our study was significantly affected by growing system. A high level of invert and total sugar content was recorded in fruits obtained in protected area, while a lower level was recorded in open field. Interestingly, the fruits obtained from the open field had two-fold higher sucrose content (1.03%) related to the fruits from the plastic-greenhouse (0.52%).

The amounts of sugars and organic acids as well as their relationship are key quality index, determining fruit taste. Their appearances and concentrations, especially their relative ratio, in fruits and berries always determine the final quality of the products derived (Zheng et al., 2019). The sugar/organic acid ratio of strawberries grown in protected area and open field in our study were 6.90 and 5.53, respectively.

Generally, the highest TSS, TS and TA level as well as TS/TA ratio and therefore, a better taste of the fruit was detected in samples of cultivar 'Senga Sengana' grown under plastic-greenhouse (Table 4).

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Table 4. Chemical composition of strawberry cultivar 'Senga Sengana'

	Growing system	
	Protected area	Open field
TSS (°Brix)	9.30±0.12 a	7.10±0.12 b
IS (%)	5.45±0.03 a	3.06±0.02 b
SUC (%)	0.52±0.02 b	1.03±0.01 a
TS (%)	6.00±0.00 a	4.15±0.04 b
TA (%)	0.87±0.02 a	0.75±0.01 b
TS/TA	6.90±0.01 a	5.53±0.03 b
pH	3.46±0.01 a	3.45±0.02 a

TSS – total soluble solids; IS – invert sugars; SUC – sucrose; TS – total sugars; TA – total acids; TS/TA – sugars/acids ratio.
The different lower-case letters in the columns indicate statistically significant differences among cultivars at P≤0.05 (Duncan's test).

Numerous studies have proven that daily consumption of berries affects the improvement of human health. The healing properties of the fruit are explained mainly by the presence of secondary metabolites, which have different biological activities. Strawberries generally possess a high level of antioxidant activity, which is linked to the levels of pigments and phenolic compounds in fruits (Wang and Jiao, 2000; Vinson et al., 2001; Sun et al., 2002; Mandave et al., 2014). In addition to phenolic compounds, vitamin C significantly contributes to the antioxidant activity of strawberry fruits. Many factors such as genotype, soil type, light, temperature, applied agricultural techniques affect the phenols and anthocyanin content of plants (Hosseinian et al., 2007). Analysis of variance revealed a significant influence of the growing system on the content of total phenols, anthocyanins, vitamin C and antioxidant activity in strawberry fruit (Table 5).

Table 5 - Total anthocyanins, total phenols, vitamine C and antioxidative activity of strawberry cultivar 'Senga Sengana'

	Growing system	
	Protected area	Open field
Total antocyanins (mg C3G/100 g FW)	58.32±3.41 a	36.26±1.90 b
Total phenols (mg TE/100 g FW)	277.80±10.92 a	223.32±17.08 b
Vitamin C (mg/100 g FW)	41.12±2.03 a	33.10±1.15 b
Antioxidant activity (%)	72.92±0.95 a	59.74±1.50 b

The different lower-case letters in the columns indicate statistically significant differences among cultivars at P≤0.05 (Duncan's test).

The content of vitamin C, anthocyanins and total phenols in strawberry fruits from plastic-greenhouse were significantly higher, which caused a significant increase in antioxidant capacity in the fruit compared to open field condition. In addition to the positive impact of the protected area on the sensory and nutritional quality of the fruit, it is important to note that this growing system ensures safe yields, bearing in mind that growing strawberries in a plastic-greenhouse prevents damage to flowers from spring frost, and greatly reduces the appearance of gray mould.

Conclusion

Based on obtained results, it can be concluded that the right selection of the growing system can significantly affect the quality of the strawberry fruit. Changes that occurred in the yield, physical and chemical properties of strawberry fruits depending on the growing system indicate that the best results were achieved in a protected area. The high sensory (soluble solids, sugars, acids, sweetness index) and nutritional quality (vitamin C, anthocyanins, phenols) of the fruit in the cultivar 'Senga Sengana' indicate a justifiably high presence of this cultivar in strawberry plantations in Serbia as well as its primary purpose for processing.

However, in addition to the expansion of growing strawberries in a protected area, it is necessary to innovate the strawberry assortment intended for processing. It is necessary to introduce new cultivars suitable for growing in the open field and protected area, which are resistant to diseases and gives high yields of quality fruits, in order to ensure economic production, especially bearing in mind that the yield per plant of strawberries less than 500 g is not economical.

Acknowledgement

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INFLUENCE OF MACERATION CONDITIONS ON THE ANTIOXIDATIVE PROPERTIES OF VRANAC AND MERLOT RED WINES

Tatjana Jovanović-Cvetković[1], Aleksandar Savić[2], Danijela Starčević[1], Boris Pašalić [1]

Abstract: Maceration is the basic process by which red and white wine production technologies differ from each other, and thanks to this process, red wines acquire most of their characteristics. Mainly during maceration, phenolic compounds, which are powerful antioxidants, are extracted from the solid parts of the berries. Depending on the variety, it is necessary to apply different maceration regimes. The aim of this research was to determine the influence of the maceration temperature and duration on the antioxidant properties of red wines of the Vranac and Merlot varieties. The research was carried out in 2021, where maceration procedures were applied for 6 and 12 days, at temperatures of 16 and 25°C. When macerated for 6 days at 25°C, the wines of both varieties had the lowest total phenolic content (Vranac 7.27 g/l; Merlot 7.15 g/l), but the highest total free anthocyanin content (Vranac 841.04 mg/l; Merlot 572.85 mg/l). Higher antioxidant activity of Vranac variety wines was obtained at shorter maceration (6 days), and Merlot variety wines at longer maceration (12 days).

Key words: maceration, phenolic compounds, anthocyanins, antioxidant activity

Introduction

Maceration is an essential part of the technological process of red wine production (Ribéreau-Gayon et al., 1970). The process of maceration implies the extraction of certain compounds from the solid parts of cluster and berries, and their transition into the liquid phase, i.e. wine (Blesić et al., 2013). Primarily, during maceration, phenolic compounds (tannins and colored substances), and also a number of other substances important for wine quality are separated from the solid parts, (Ribéreau-Gayon et al., 2006). Over 85% of phenolic

[1]University of Banja Luka, Faculty of Agriculture, Bulevar Vojvode Petra Bojovića 1A, Banja Luka, Republic of Srpska (tatjana.jovanovic-cvetkovic@agro.unibl.org)

[2] University of Banja Luka, Faculty of Technology, Bulevar Vojvode Stepe Stepanovića 73, Banja Luka, Republic of Srpska (aleksandar.savic@tf.unibl.org)

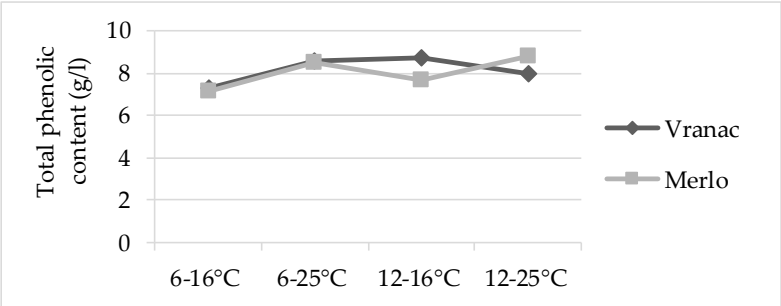
compounds are flavonoids, and among them, anthocyanins and tannins stand out with their importance for the grape and wine quality (Blesić, 2016). In addition to contributing to the taste and quality of wine, phenolic compounds have a strong antioxidant effect on human health (Beer, 2002). There is a great number of variables that affect the maceration process, but duration and temperature have the greatest impact (Waterhouse et al., 2016). The extraction of phenolic compounds is significantly accelerated by increasing the temperature (Merida et al., 2015). By increasing the temperature, the permeability of the grapes' skin cells also increases, which is the main reason for greater extraction of anthocyanins, and also other phenolic compounds (Şener, 2018). The most intense anthocyanin extraction takes place between the fourth and fifth day and then gradually decreases, so macerations longer than 10 days do not lead to a significant increase in anthocyanin extraction (Waterhouse et al., 2016). During long-term maceration, the tannins are extracted into the wine, and such wines mostly have a good ageing potential (Blesić 2016). The aim of the research was to determine the influence of the duration and temperature of maceration on the properties of red wines of the Vranac and Merlot varieties.

Material and methods

The research was carried out in 2021. Grape samples were obtained from the "Dabić" winery located in the village Bihovo, near Trebinje. The analysis included two varieties: Vranac and Merlot. Microvinification was carried out in the Microbiology Laboratory of the Faculty of Technology in Banja Luka. Maceration procedures were applied for 6 and 12 days, at temperatures of 16 and 25°C. After the alcoholic fermentation, the obtained wines were analyzed in the Food Analysis Laboratory of the Faculty of Technology of the University of Banja Luka. Measurements were made in 2 repetitions, and the following analyzes were made: a) determination of total phenolic content (TPC) – modified spectrophotometric method (Wolfe et al., 2003); b) determination of anthocyanin content – spectrophotometric method according to Glories (1984); c) determination of total antioxidant activity - FRAP (ferric reducing antioxidant power) is determined according to the method developed by Benzie & Strain (1996); d) determination of antioxidant properties by the DPPH method according to Liyana-Pathiranana & Shahidi (2005) and by the ABTS method, also called the TEAC method in the literature (Re et al., 1999).

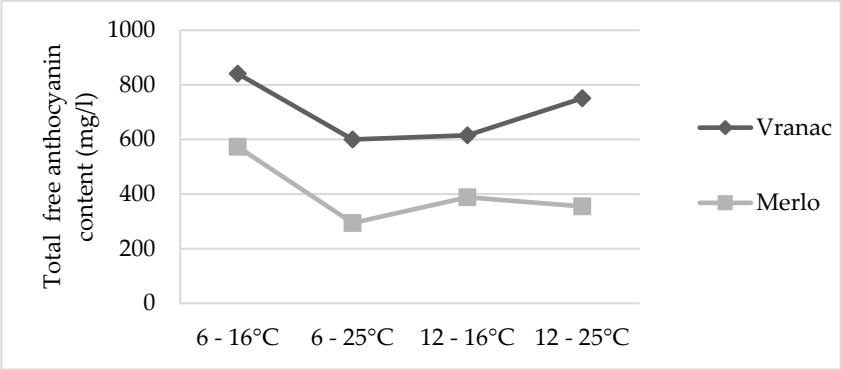
Results and discussion

Since phenolic compoun ds are powerful antioxidants, the total phenolic content in wine (graph 1.) is very important when evaluating the antioxidant properties of wine.



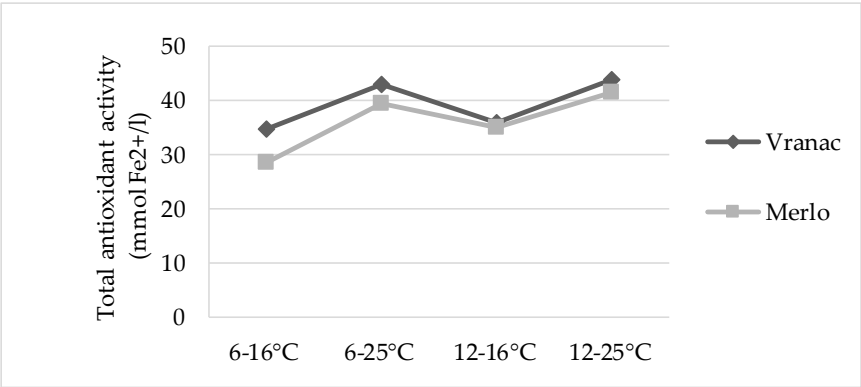
Graph 1. Total phenolic content in Vranac and Merlot wines at different maceration regimes

After 6 days of maceration at 25°C , Merlot wines had total phenolic content of 8.46 g/l, while longer maceration at the same temperature additionally increased the total phenolic content (8.82 g/l). Wines of the Vranac variety had the highest total phenolic content during maceration for 6 days at 25°C (8.58 g/l) and 12 days at 16°C (8.69 g/l). The lowest total phenolic content in the wines of both varieties was observed after 6 days of maceration at a temperature of 12°C. According to Pajović et al. (2011) at maceration temperatures ranging from 22 to 24°C, less total phenols were isolated in Vranac wine, than at maceration temperatures of 28-31°C. According to Ivanova et al. (2009) wines of the Merlot variety had the highest total phenolic content when maceration lasted for 10 days (3176–3467 mg/l). The anthocyanin content in wine (graph 2.) mostly affects its color and antioxidant properties.



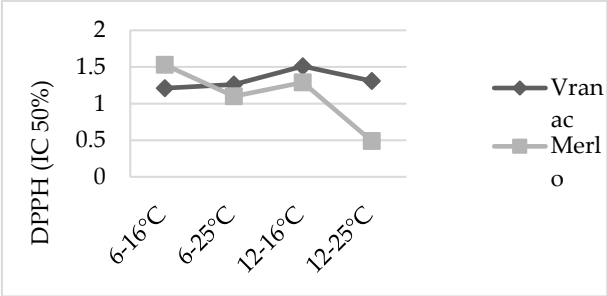
Graph 2. Total free anthocyanin content in Vranac and Merlot wines at different maceration regimes

The wines of both analyzed varieties had the highest total free anthocyanin content after 6 days of maceration at 16 °C (Vranac 841.04 mg/l; Merlot 572.85 mg/l), and the lowest after 6 days of maceration at 25°C (Vranac 599 .85 mg/l; Merlot 294.12 mg/l). According to Milanov et al. (2014) in the wines of the Vranac variety, the content of total anthocyanins was higher when the maceration lasted for 7 days (up to 582.25 mgL-1) compared to the wines where the maceration was extended to 14 days (405–447 mgL-1). In Merlot wine, according to Ivanova et al. (2009) the highest anthocyanin content was observed in wines macerated for 6–10 days (496–506 mg/l). The antioxidant activity of wine was determined through FRAP (chart 3), DPPH (2,2-diphenyl-1-picrylhydrazyl) (chart 4) and ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) (graph 5.) tests.

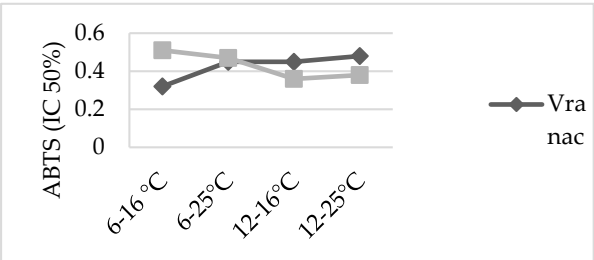


Graph 3. Total antioxidant activity of Vranac and Merlot wines at different maceration regimes

A higher concentration of Fe2+/l represents a higher antioxidant activity of the wine. Temperatures of 25°C increased the antioxidant activity of all analyzed wines. The highest antioxidant activity for wines of both varieties was obtained after 12 days of maceration at 25°C (Vranac 43.89 mmol Fe2+; Merlot 41.52 Fe2+). The lowest antioxidant activity was measured after 6 days of maceration at 12°C.



Graph 4. DPPH values in Vranac and Merlot wines at different maceration regimes



Graph 5. ABTS values in Vranac and Merlot wines at different maceration regimes

The obtained results of the DPPH and ABTS tests are expressed as IC 50% (concentration required to scavenge 50% of the radicals). Lower IC 50% concentrations represent a stronger antioxidant effect of wine. Vranac variety wines had the lowest value of DPPH after 6 days of maceration (IC 50% - 1.21

and 1.26), and the highest after 12 days of maceration at 16°C (IC 50%=1.51). In Merlot variety wine, the lowest antioxidant activity was measured after 6 days of maceration at 16°C (IC 50%=1.53), and the highest when maceration was carried out for 12 days at 25°C (IC 50%=0.49). Wines of the Vranac variety had the lowest ABTS value after 6 days of maceration at 16°C (IC 50%=0.32), while in the case of the Merlot variety, longer maceration (12 days) had a positive effect on the reduction of the ABTS value (IC 50% - 0, 36 and 0.38).

Conclusion

The duration and temperature of maceration influenced the production of wines with differen+t antioxidant properties. Longer maceration has led to increase in total phenolic content in wines of the Merlot variety, while in the wines of the Vranac variety, after 12 days of maceration, there was a noticeable decrease in the total phenolic content. Wines of the Vranac variety had higher antioxidant activity during shorter maceration, while the antioxidant activity of Merlot wines was higher during longer maceration. Increasing the maceration temperature from 16°C to 25°C had a positive effect on the antioxidant properties of the wines of both varieties.

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THE INFLUENCE OF CORN PRICE IN THE CURRENT YEAR ON THE PIGS NUMBER AND FATTENING PRICE OF THE FOLLOWING YEAR

Nebojša Novković[1], Veljko Šarac[1], Nataša Vukelić[1], Dragana Tekić[1], Beba Mutavdžić[1]

Abstract: The aim of the research is to determine relationship between the price of corn in the current year and the number and price of fattening pigs in the following year. The analysis was done for the period from 2006-2021. Descriptive statistics were used for data processing. To determine the impact of corn price on the price and number of fattening pigs, a regression model was applied. A moderate variability of the observed parameters was determined, with increasing of corn price at a rate of 7.92% per year, fattening pigs 3.88%, and the number of fattening pigs decreasing by rate of 1.75%. The results of the regression analysis indicate a significant impact of the corn price on the number and price of fattening pigs.

Keywords: price, corn, fattening pigs, state, tendencies.

Introduction

The importance of livestock production in the economy of a country is highlighted in many ways. Zekić et al., (2007) point out that due to the shorter time of the production process, livestock production contributes to the overall growth rate of agricultural production. In addition, pig farming as a branch of livestock production is characterized by Mirilović et al., (2012) as its largest and most important part. Also, the autors belive that the development of pig farming as an economic activity is important for the development of the country's economy as whole. In the structure of livestock production, all production lines has decline, except pig production which grew by 0.8% (Statistical yearbook 2021). In the European Union (EU), pig production has a large contribution, where in 2018 the sector of pork production participated with 9% in total agricultural production, and with 35% in the total meat production of EU (Renaudeau and Dourmad, 2022). One of the biggest sources of food for livestock production is plant production. The most dominant share

[1] Universtiy of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, Novi Sad, Serbia (nebojsa.novkovic@polj.uns.ac.rs)

is realized by the corn production line, because the largest part of the produced corn is used for animal feed preparation (Radosavljević, 2007). The subjects of this research are the price, number of fattening pigs and the price of corn. The aim of the research is to determine the impact of corn price from current year on the price and number of fattening pigs in the next year. The hypothesis of this research is that the price of corn has a statistically significant impact on the price and number of fattening pigs.

Materials and methods

The research was conducted using standard quantitative methods. The collected data were processed with statistical tools: average value $\overline{X}$, extreme values (min, max), coefficient of variation (CV) and annual rate of change in % (r). According to the aim of the research, a linear regression model was used to determine the impact of the corn price in the current on the pigs number and fattening price of following year. Regression analysis is a method used to identify relationships between observed variables (Munćan and Božić, 2018). Additional analysis determines the direction and strength of the identified connections. Also, regression analysis can be defined as an assessment of the value of the dependent variable on one or more independent variables (Mutavdžić and Đorić, 2018). The general form of the regression model is:

$$\hat{Y}_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \cdots + \beta_p X_{pi} + \varepsilon_i$$

Where $\hat{Y}_i$ represent the value of the dependent variable, X_{1i}, X_{2i}, … , X_{pi} represent the value of independent variable, and β_1, β_2, ….. β_p, represent the regression parameters. The parameter β_0, shows the average level of the dependent variable, while ε_i is the random error of model (Novaković, 2019). To check the statistical significance of the defined model, a regression variance analysis was performed. The data used in analysis were taken from the website of the Republic Institute of Statistics. The data refer to the corn price, the price of fattening pigs, as well as the number of fattening pigs in the Republic of Serbia for the period from 2006-2021. The IBM STATISTICS 21 software package was used for statistical data processing.

Results and discussion

The basic indicators of descriptive statistics for the observed parameters of corn and fattening pigs prices, as well as the number of fattening pigs are shown in the following table (Table 1).

Table 1. Descriptive statistics of observed parameters in the period 2006-2021.

Product	Parameter	Average	Min	Max	CV (%)	Rate of change (%)
Corn	Price (din kg^{-1})	14.70	7.47	23.43	27.41	7.92
Fattening pigs	Price (din kg^{-1})	140.30	73.06	176.89	21.22	3.88
	Number (000)	1,432.56	1,301.00	1,831.00	9.30	-1.75

Source: Author's calculation.

In the analyzed period at the territory of the Republic of Serbia, the average corn price was 14.70 din kg-^{1}, while it ranged from 7.47 to 23.43 din kg-^{1}. Also, the corn price has medium variation CV=27.41%. The average fattening pigs price was 140.30 din kg-^{1}, while the minimum price was 73.06, and the maximum 176.89 din kg-^{1}. The price of fattening pigs recorded a lower, but still pronounced variability (CV=21.22%). The total number of fattening pigs in the observed period averages 1,432.56 thousand heads. The number of fattening pigs shows moderate variability, which is supported by the significantly lower coefficient of variation 9.30% in relation to the corn and the fattening pigs price. The number of fattening pigs varied in the interval of minimum 1,301.00 to a maximum of 1,831.00 thousand heads. The obtained results of descriptive statistics indicate a negative tendency, a decrease in the number of fattening pigs in the analyzed period at an annual rate of 1.75%. Despite this, the interest of producers is found in a positive trend, a growth in the price of fattening pigs of 3.88% on an annual basis. However, the decline in the number of fattening pigs during the period can be justified by the increase in the price of one of the most important inputs for the preparation of feed mixtures, corn, whose annual price increases at a rate of 7.92%, which is in line with the results obtained by Novković et al., (2019), where authors predict the growth of corn prices in the future using a group of ARIMA models. In the period from 2001-2012, according to Mirilović et al., (2015) the number of fattening pigs decreases annually by 4,248.00 heads, while the price of corn and the price of fattening pigs tends to increase in the territory of the Republic of Serbia, which is in accordance with the indicators obtained for the time interval covered by the research (2006-2021).

In the next part of the research, based on the variables, the regression model 1. was formed, for determining the dependence of the fattening pigs price in the next year from the price of corn in the current year. The model was tested as a whole using analysis of variance regression (Table 2). The hypothesis for testing the model as a whole is: $H_0: \beta_1 = \beta_2 = \cdots = \beta_k = 0$, and if it is accepted, it is considered that the model is not statistically significant.

Table 2. Analysis of variance for the regresion model 1.

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	10,623.130	1	10,623.130	55.668	0.000
	Residual	2,671.624	14	190.830		
	Total	13,294.754	15			

Source: Author's calculation.

The obtained results of variance of the regression analysis for the regression model 1. indicate the significance of the model, the null hypothesis is rejected and it is concluded that the selected model is statistically significant ($p<0.05$). The estimated parameters of regression model 1., dependence of fattening pigs price in the next year in relation to the price of corn in the current year are shown in Table 3.

Table 3. Regression model 1. Influence of corn price on fattening pigs price.

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig
		b	Std. Error	Beta		
1	Constant	44.909	13.244		3.391	0.004
	Corn price	6.995	0.938	0.894	7.461	0.000

Source: Author's calculation.

Estimated regression model: $\hat{Y} = 44.909 + 6.995X_1$.

The regression coefficient (b) confirms the statistical significance of the regression parameter (corn price in current year). The influence of the independent variable has a positive character. It is concluded that the increase in the price of fattening pigs is influenced by the increase of corn price. If the corn price increases by 1 din kg^{-1} in the current year, an increase in the price of fattening pigs of 6.995 din kg^{-1} can be expected in the next year. In the regression model 2., the independent variable is corn price in the current year, and the dependent variable is the number of fattening pigs in the next year. The model was tested as a whole using analysis of variance regression (Table 4).

Table 4. . Analysis of variance for the regresion model 2.

Model		Sum of Squares	df	Mean Square	F	Sig.
2	Regression	70,720.245	1	70,720.245	5.057	0.041
	Residual	19,801.693	14	13,985.835		
	Total	266,521.938	15			

Source: Author's calculation.

The results of the variance analysis of regression model 2. point to the conclusion that the null hypothesis is accepted and that the selected model is statistically significant ($p<0.05$). There is a statistically significant relationship between corn price in current year and the number of fattening pigs in the following year. The estimated parameters of regression model 2., the dependence of the movement of fattening pigs number in the next year in relation to the corn price in the current year are shown in Table 5.

Table 5. Regression model 2. Influence of corn price on fattening pigs number.

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig
		b	Std. Error	Beta		
2	Constant	1,678.70	113.379		14.806	0.000
	Corn price	-18.048	8.026	-0.515	-2.249	0.041

Source: Author's calculation.

Estimated regression model: $\hat{Y} = 1{,}678.70 - 18.048X_1$.

A statistically significant influence of the current year's corn value on the number of fattening pigs in the following year was determined. A negative value of the regression parameter was found in the model (b). If the price of corn increases in the current year, the number of fattening pigs is expected to decrease in the following year. The evaluated regression model leads to the conclusion that if corn price increases by 1 din kg^{-1}, the number of fattening pigs is expected to decrease by 18.048 thousand heads in the next year. The limitation that occurs in both formed models refers to the inclusion of only one independent variable in the model (corn price). By including a larger number of independent variables, with the assumption of the significance of the model as a whole, the level of reliability of the obtained results increases, but we should also have in mind the opinion of Nikolić-Đorić et al., (1993) that complexity of the model does not necessarily lead to its reliability.

Conclusion

During the observed period from 2006 to 2021., at the territory of the Republic of Serbia, the fattening pigs price is increasing at an annual rate of 3.88%, while the total number of fattening pigs decreasing at rate of 1.75%. The average fattening pigs price is 140.30 din kg^{-1}, while average number is 1,432.56 thousand heads. The price of corn is growing at annual rate of 7.92%, at an average value of 14.70 din kg^{-1}. According to the aim of the research, regression models 1. and 2. were formed. The obtained result of the regression models

concludes a statistically significant influence of corn price from current year on the fattening pigs price and number of the following year. Increasing of corn price by 1 din kg^{-1}, there is an increase of fattening pigs price by 6.995 din kg^{-1}. By increasing of corn price by 1 din kg^{-1}, there is a decrease of fattening pigs number by 18.048 thousand heads. In both cases, the initial hypothesis about the significance of the impact of corn price on the price and number of fattening pigs were confirmed.

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INSIGHTFUL APPLICATION OF HERBAL EXTRACTS IN THE PREVENTION AND TREATMENT OF ANIMAL DISEASES AND IMPROVEMENT OF MEAT QUALITY AND SAFETY

Vladimir Kurćubić[1], Slaviša Stajić[2], Nemanja Miletić[1], Marko Petković[1]

Abstract: In an indirect way, through animal nutrition or selection, we can influence the composition and quality of muscle and fat tissue, and thus the nutritional and functional value of meat. The process is longer and is not fully controlled yet. Direct processes improve nutritional properties by adding components with functional properties and/or reducing or removing ingredients that may negatively affect health. Herbal raw materials and extracts, by-products and wastes from fruit and vegetable processing can modify feed in order to achieve a stable health status and preserve animal well-being and sensory quality of meat, with an extended shelf life.

Key words: herbal extracts, antimicrobial activity, animal health, fodder, meat quality

General considerations on modern concepts

In recent years, an alarming prevalence of multiresistant microorganisms has been observed as a consequence of the uncontrolled use of antibiotics in metaphylaxis in veterinary medicine or therapy in human and veterinary medicine. A number of studies have been designed with the aim of evaluating the effect of adding a mixture of plant extracts to animal feed on productive performance, blood constituents, carcass characteristics, percentage ratio of organ weight to live weight, meat sensory properties and overall quality (Dávila-Ramírez et al. 2020; Chen et al., 2021; Song et al., 2022; Xu et al., 2022; Zhang et al., 2022).

The increasing occurrence of dangerous infections caused by bacteria that are resistant to antibiotics of the latest generations (multidrug resistant - MDR) has made the research of new molecules in the field of medicine current on a

[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia. (vkurcubic@kg.ac.rs)

[2]University of Belgrade, Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade, Serbia.

global level. Compared to chemically synthesized substances, herbs provide greater structural diversity and offer more opportunities for the identification of new antimicrobial (AM) compounds. Plants show excellent antibacterial effect due to their safety, efficacy, synergism and reduced drug resistance. The combination of herbal and chemical AM agents (of synthetic origin) for the treatment of infectious diseases is popular in clinical practice in China because of their synergistic or potentiating effects (Song et al., 2022).

Numerous reasons limit the use of antiviral (AV) drugs in human and veterinary medicine, which is not the case with the use of natural AM agents (Antimicrobial Drugs - AMD). Limitations are due to the appearance of virus mutations or new viruses, toxic effects, the intensity of viral diseases, the ability of the virus to survive intracellularly, the high price and the absence of specific AV chemical preparations against pathogenic microorganisms that cause diseases in veterinary medicine. The low effectiveness of AV agents requires the discovery of new powerful AV substances. Most AV substances important in veterinary medicine are still used thanks to trials of AV drugs for human use in animal models (eg, a surrogate model for hepatitis C virus trials is Bovine Viral Diarrhoea Virus - BVDV).

A review by Karásková et al. (2015) points to the fact that the possibilities of using phytogenic additives are different. Their use does not carry great dangers, as for example the use of antibiotics or chemical compounds. Phytogenic additives and their wider practical application will undoubtedly be the subject of further research: sensory, technological, zootechnical, immunomodulation, improve production characteristics or the quality of animal products, reduce the negative effects of stress.

Information on the possible applications of phytogenic additives (probiotics, prebiotics, enzymes, plant extracts) in modern animal feed production is provided in the chapter by Pandey et al. (2019). A study by Ceruso et al. (2020) evaluated the antibacterial activity of about 800 plant extracts against L. monocytogenes. The results revealed that 12 plant extracts had an inhibitory effect against L. monocytogenes - new preservatives to reduce the risk of developing various pathogens and food contamination with L. monocytogenes. The most important reformulations of animal feed using plant-based supplements and their impact on meat quality parameters, which were shown in the world literature during the last two years (2020 - 2022), presented tabularly in recent review by Kurćubić et al. (2022).

One of the insurmountable challenges for the application of many phytochemicals is tracing efficient pathways, which can release the active

antimicrobial (AM) compound at the target site during systemic infections. The dilemma is how to select compounds that have AM activity in complex mixtures such as extracts and essential oils and how to use their potential pharmacological interactions most effectively and efficiently. For this purpose, it is necessary to use modern technologies, AM tests with internationally recognized standardized protocols and the use of herbal material with appropriate quality controls (Álvarez-Martínez et al., 2021).

A very useful scientific opinion created by experts from the Panel for additives and products/substances used in animal feed, at the request of the European Commission (EFSA FEEDAP, 2020). EFSA were asked to provide a conclusion on the safety and efficacy of the dried aqueous ethanolic extract of lemon balm (Melissa officinalis L.) leaves when used as a sensory feed additive for different animal species. The toxicity and genotoxicity of the identified components of herbal extracts are not at a worrying level. However, the analysis of the extracts is incomplete. In the absence of adequate data on the composition and in view of the incomplete genotoxicity testing, FEEDAP was not able to publish a definitive conclusion on the safety of the additive for the target species of animals and the health of consumers. Lemon balm is a European plant species and its use in animal feed is not expected to pose a risk to the environment. Lemon balm and its extracts flavor food, and the function in animal feed is the same as in human feed, so further confirmation of the effectiveness of the extract is not required.

Antibiotics, disinfectants and chemotherapeutic agents used for the prevention and treatment of diseases lead to the appearance of antibiotic and chemical residues in fish products, multi-resistant microorganisms resistant to antibiotics and damage to the aquatic environment and human health. This situation has led researchers to use alternative additives in fish nutrition such as: medicinal herbs, plant extracts, phytochemicals, and secondary metabolites of plants, immunostimulators and probiotics. A recent review (Yılmaz et al., 2022) includes research conducted in Turkey between 2001 and 2020 to summarize findings regarding the use of medicinal plants, plant extracts, phytochemicals, plant secondary metabolites, and immunostimulants in fish feed for the prevention and treating diseases, improving immunity, increasing disease resistance and reducing stress in fish towards better management and best practices in aquaculture for the sustainability of the growing aquaculture industry in the region and around the world.

Kurćubić et al. (2013) determined the effectiveness of 0.3% ethanolic herbal extract of the Kitaibelia vitifolia applied as a spray, lasting 5 seconds, to destroy

E. coli on beef carcasses. The strong AM action of the ethanolic extract of the plant K. vitifolia applied as a spray on the reduction of the number of E. coli (Enterobacteriaceae), which was applied as an inoculum in a known number to the surface of the meat, was confirmed.

Endophytes (microorganisms that live in plant tissues without typically causing any harmful effects on them on a commercial scale) could progressively eliminate our direct dependence on high-value vulnerable plants, thus opening a sustainable way to use plant resources in a sustainable manner (Sharma et al., 2021).

The prevention of respiratory diseases in cattle as the most economically significant health disorder implies that each control program must be designed by experts as multicomponent. That includes the use of different vaccines and types during vaccination/revaccination, the use of antimicrobial drugs (AMD) upon arrival of calves in facilities (feedlots), biosecurity measures, diagnostic procedures for determining the occurrence of the disease and its treatment, breeding practices, testing the quality of animal feed and nutrition programs, monitoring and intervention programs for the preservation of animal health (Ives and Richeson, 2015; Brault et al., 2019; Lhermie et al., 2019). The synergistic or potentiating effect of herbal AMD and chemical AMD is embodied in clinical practice. Tulathromycin and gallic acid combined were very effective against M. haemolytica, P. multocida and their mixed cultures. Separately, they had a very weak effect on both mentioned bacteria. Tulathromycin pre-exposure generates bacterial resistance to AMD in M. haemolytica but not in P. multocida (Rajamanickam et al., 2019).

An attempt to check in tissue culture the antiviral effect of plant extracts on those viruses that cause Bovine Respiratory Disease Complex (BRDC), proven to be the most economically significant syndrome of fattening steers and cattle worldwide, in general, in Serbia, was carried out by Kurćubić et al. (2019). They investigated the in vitro AV activity of 5 selected plant extracts against Bovine Herpes Virus-1 (BHV-1) subtype 1.1 and Bovine Viral Diarrhea Virus (BVDV) genotype 1, which together with other viral and bacterial agents cause BRDC. Two prepared samples of aqueous plant extracts/macerates showed an antiviral (AV) effect against BHV-1 as a representative of herpesvirus (DNA virus), but none of the tested samples showed an AV effect against BVDV genotype 1 strain NADL, which belongs to RNA viruses. In this test, the Selectivity Index (SI = CTC50/EC50) for aqueous extracts of Matricaria chamomilla (SI = 32) and Achillea millefolium (SI =≥ 8) indicated significant selective inhibition of DNA virus (BHV-1, strain TN41). The obtained results require new and significantly

more extensive research, which would reveal the active ingredients of the examined plants and the mechanisms of their AV effect.

Due to the need to protect the efficacy of significant AMDs for use in human medicine, WHO has published guidelines presenting evidence-based recommendations and best practice statements on the use of "medically important antimicrobials" in animal feed, defined as AM classes that used in human medicine (WHO, 2017). Medically important AMDs are categorized according to appropriate criteria as "important", "extremely important" or "critically important" for human medicine. WHO recommends that the overall use of medically important AMD in animal feed be reduced. Their use for growth promotion in animals not diagnosed with clinical disease is prohibited.

Conclusion

In the future, studies on the AM power of plants will be designed through understanding the mechanisms of action, pharmacodynamic basis and pharmacokinetics. The WHO recommendations that critical AMD be used only for the treatment of certain animal diseases, and for the group of "critically important" AMD of the highest priority, use as a feed additive are prohibited, seem very reasonable.

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THE EFFECT OF THE REARING SYSTEM AND GENOTYPE OF LAYING HENS ON FATTY ACID COMPOSITION OF EGGS

Simeon Rakonjac[1], Snežana Bogosavljević-Bošković[1], Vladimir Dosković[1], Miloš Lukić[2], Zdenka Škrbić[2], Veselin Petričević[2], Milun D. Petrović[1]

Abstract: This paper aimed to determine the effect of the rearing system and genotype of laying hens on the fatty acid composition of eggs. Seven times during the one-year production cycle, 15 eggs from all four experimental groups were taken, and fatty acid composition was determined on pooled samples. Based on the obtained results, it can be concluded that the highest content of SFA had organic New Hampshire eggs, MUFA organic Isa Brown eggs and PUFA floor-produced eggs. Regarding the ratio of n-6:n-3 PUFA, it can be noted that the organic eggs had a lower ratio compared to the floor eggs, as well as the New Hampshire breed compared to the Isa Brown hybrid.

Keywords: laying hens, floor system, organic system, breed, hybrid, fatty acid.

Introduction

Omega-3 fatty acids have a wide range of demonstrated health-related benefits. These positive effects include: cardioprotective, anticancer, triglyceride and blood pressure lowering, immune health enhancing and their roles in the growth and maturation of the central nervous system (Cherian and Quezada, 2016), and also prevention of some psychiatric disorders, from stress to depression and dementia (Bourre, 2005). Special attention is paid to the content of omega-3 (n-3) and omega-6 (n-6) fatty acids, as well as their ratio, so Simopoulos (2000) report that the ideal ratio of n-6 to n-3 is 10:1 and lower, and Johansson (2010) 4:1 and lower. Samman et al. (2009) state that people consuming n-3 fatty acids from the so-called "engineered eggs" reduce the risk factors of heart diseases, and Johansson (2010) that the most common diseases in western countries, such as cardiovascular problems, diabetes, cancer, autoimmune diseases, are related to an inappropriate ratio of n-6:n-3 polyunsaturated fatty acids. During the long evolutionary history of *Homo*

[1]University of Kragujevac, Faculty of Agronomy in Čačak, Cara Dušana 34, Čačak, Serbia (simeonr@kg.ac.rs)

[2]Institite for Animal Husabandry Belgrade-Zemun, Autoput 16, Poštanski fah 23, 11 080 Belgrade-Zemun, Serbia

sapiens, an n-6:n-3 fatty acid ratio balance has existed, and it is believed that many genetic changes happened partly because of it (Nyberg, 2017).

For this reason, this paper aimed to determine the effect of the rearing system and genotype of laying hens on the fatty acid composition of eggs.

Materials and methods

The experiment was arranged in a 2 x 2 factorial design with two layer genotypes (Isa Brown hybrid and New Hampshire breed) and two rearing systems (floor and organic). 30 birds per group were housed at 18 weeks of age.

In both rearing systems, stocking density was 2.5 birds/m^2. Organic layers also had about 5 m^2 per bird of available outdoor area covered with grass and bushes, which enabled them to supplement their diets using vegetation and small creatures living outdoors.

Floor-reared laying hens were fed *ad libitum* a standard commercial diet. The diet for organic hens was complete without synthetic amino acids, vitamins and minerals and with more than 80% of organic components.

In order to examine the effect of the rearing system and genotype on the fatty acid composition of eggs, seven times during the one-year production cycle (24, 32, 40, 48, 56, 64 and 72 weeks hen's age), 15 eggs from all four experimental groups were collected, homogenized, and the fatty acid composition (pooled samples) was determined by HPLC. The analysis of the obtained data was performed based on calculated mean values using the computer program Statistica (Stat Soft Inc Statistica for Windows. Version 7.0., 2006).

Results and discussion

Table 1 shows the content of the most important fractions of fatty acids and their ratio.

The highest content of saturated fatty acids (SFA) in eggs during the experimental period in five controls was recorded in the organic New Hampshire group. Regarding the minimum content of SFA in eggs, in five of seven controls it was recorded in the floor experimental groups, three in Isa Brown (at 24, 56 and 72 weeks), and two in the New Hampshire group (in 40 and 64 weeks).

Table 1. Fatty acid composition of eggs by experimental groups

Treatment	Fatty acid	Hen's age						
		24	32	40	48	56	64	72
		Fatty acid content (%) of total fatty acids						
Isa Brown floor	SFA	32.64	32.94	33.10	34.11	32.45	32.88	32.76
	MUFA	45.45	44.89	49.06	45.25	40.38	42.64	44.21
	PUFA	20.25	20.58	16.57	19.17	25.73	22.96	21.71
	n-3	1.12	1.24	0.79	0.97	1.68	1.70	1.25
	n-6	19.13	19.33	15.78	18.20	24.05	21.26	20.45
	n-6/n-3	17.05	15.53	20.04	18.78	14.31	12.51	16.31
New Hampshire floor	SFA	34.06	33.34	32.99	33.74	32.34	32.77	32.90
	MUFA	42.26	42.89	42.31	46.54	45.32	42.32	41.52
	PUFA	22.13	22.20	23.06	18.43	20.85	23.30	23.98
	n-3	0.88	1.62	1.87	0.68	1.30	2.22	2.15
	n-6	21.25	20.58	21.19	17.75	19.55	21.08	21.82
	n-6/n-3	24.14	12.74	11.33	26.04	15.05	9.48	10.14
Isa Brown organic	SFA	34.97	30.91	33.48	32.86	36.29	32.84	32.89
	MUFA	48.60	50.12	50.05	48.80	40.53	43.09	44.79
	PUFA	14.49	18.09	14.56	16.88	21.08	22.37	20.41
	n-3	1.01	1.09	0.71	1.02	1.81	1.70	1.71
	n-6	13.48	17.00	13.86	15.86	19.27	20.67	18.70
	n-6/n-3	13.36	15.60	19.65	15.59	10.66	12.17	10.94
New Hampshire organic	SFA	34.26	36.11	34.94	34.36	34.11	33.52	34.08
	MUFA	45.27	42.99	42.90	46.34	42.44	43.04	43.38
	PUFA	18.29	18.98	20.55	17.37	21.59	21.57	20.95
	n-3	1.41	1.41	1.77	1.38	1.51	1.86	1.59
	n-6	16.88	17.57	18.77	15.98	20.08	19.71	19.35
	n-6/n-3	11.94	12.48	10.59	11.56	13.28	10.60	12.17

SFA - Saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids; n-3 - omega-3 fatty acids; n-6 - omega-6 fatty acids.

Regarding the content of monounsaturated fatty acids (MUFA) in eggs, in six out of seven controls, the highest content of this fraction was recorded in the organic Isa Brown group. On the other hand, the lowest MUFA content in eggs

was recorded in the floor experimental groups, at 24, 32, 40, 64 and 72 weeks in New Hampshire, and 48 and 56 weeks in Isa Brown.

The highest content of polyunsaturated fatty acids (PUFA) in eggs was recorded in the floor experimental groups - in the 24, 32, 40, 64 and 72 weeks in the New Hampshire breed, and the 48 and 56 weeks in the Isa Brown hybrid. The lowest content of PUFA in eggs was recorded in six controls in the organic groups, in 24, 32, 40, 48 and 72 weeks in the Isa Brown and the 64 weeks in the New Hampshire.

The results on the ratio of SFA, MUFA and PUFA from our research are in accordance with the results reported by Cerolini et al. (2005), who also determined a significantly higher SFA content in eggs in floor compared to organic layers, so the PUFA:SFA ratio in floor produced eggs was 0.92 and in organic eggs only 0.66. And Samman et al. (2009) found a higher SFA content in organic eggs (34.6%) compared to conventional eggs (33.8%), while MUFA and PUFA levels did not differ significantly. On the other hand, Nyberg (2017) found no significant effect of the rearing system on the SFA content in eggs, while there was a significant effect on the content of MUFA and PUFA. Pavlovski et al. (2011), Simčić et al. (2011) and Terčić et al. (2012) did not find a significant effect of the rearing system on the SFA:MUFA:PUFA ratio in eggs.

Regarding the ratio of n-6:n-3 PUFA, it can be noted that the organic experimental groups had a lower ratio compared to the floor experimental groups. The same can be said for the New Hampshire genotype compared to the Isa Brown hybrid. The lower n-6:n-3 ratio in eggs was recorded in the first four controls in organic New Hampshire layers, in the 56th week in the organic Isa Brown hens, and in the 64 and 72 weeks in the floor New Hampshire layers. The higher ratio of n-6:n-3 fatty acids in eggs was found in six out of seven controls in the floor experimental groups, in the Issa Brown at 40, 64 and 72 weeks, while in New Hampshire at 24, 48 and 56 weeks.

The lower n-6:n-3 fatty acids ratio in the organic compared to floor eggs in our research is in agreement with the results of numerous authors who found that access to and feeding on the outlet leads to an increase in the content of n-3, and a decrease in the content of n-6 fatty acids. Thus, Pavlovski et al. (2011) found that the ratio of n-6:n-3 was significantly different - 8.64:13.25, and more favourable in free range compared to cage-produced eggs. A more favourable fatty acid composition of eggs in the rearing system with outlet was also established by Simčić et al. (2011), who determined the lower n-6:n-3 ratio in free-range (6.27) compared to cage-reared hens (10.37) on the Styrian breed.

Similar results were reported by Terčić et al. (2012) on Prelux-G, while Mugnai et al. (2013) found a 4.75 n-6:n-3 ratio in organic and 8.88 in cage Ancona layers.

The most favourable ratio of n-6:n-3 fatty acids in the last two controls in floor New Hampshire layers in our experiment is not unexpected, because in that period the organic hens did not have available plants on the outlet, so there was no possibility that their eggs be enriched with an additional level of n-3 fatty acids. This was confirmed by Kucukyilmaz et al. (2012), who found a significantly higher n-6:n-3 ratio in the organic eggs of both investigated hybrids. This ratio was 12.53 in caged Lohmann LSL while organic eggs of the same hybrid had 14.28. Even more drastic differences were found in ATAK-S eggs - conventional 11.95 and organic 15.56. These results clearly show that the genotype significantly affects the n-6:n-3 ratio, which is in agreement with the results of our research, where the eggs of New Hampshire layers had a significantly better fatty acid composition than the eggs of the Isa Brown hybrid. A better n-6:n-3 ratio in the eggs of pure breed compared to hybrid was determined in research by Pavlovski et al. (2011), comparing Banat Naked Neck (5.81) and Hy-Line Brown hybrid (8.64) in a free range system.

Conclusion

Based on the obtained results, it can be concluded that the highest content of SFA had organic New Hampshire eggs, MUFA organic Isa Brown eggs and PUFA the floor produced eggs. Regarding the ratio of n-6:n-3 PUFA, it can be noted that the organic eggs had a lower ratio compared to the floor eggs, as well as the New Hampshire breed compared to the Isa Brown hybrid.

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EFFECT OF ELEVATED HEAT AND HUMIDITY ON CHEWING ACTIVITY, YIELD AND CHEMICAL COMPOSITION OF MILK IN LACTATING COWS

Blagoje Stojković[1], Bojan Stojanović[1], Nenad Đorđević[1], Vesna Davidović[1]

Abstract: The objective of this study was to investigate the influence of surrounding temperature and humidity in different periods of the year on eating and rumination time on milk yield and chemical composition in Simmental cows at late lactation (over 150 days in milk, DIM). Ambient temperature and relative humidity were registered every hour using data logger Testo 174H. Rumination time and eating time data were collected using an automatic system - GEA CowScout Neck. It was noticed that different values of THI (temperature and humidity index) affect eating time ($p<0.01$), rumination time ($p<0.01$), milk yield ($p<0.05$) as well as milk fat content ($p<0.05$). The average value of THI_1 (52.55) had a positive effect on eating time (307.2 min/day), rumination time (384 min/day), milk yield (28.97 kg/day), and milk fat content (4.25%), compared to the average value of THI_2 (72.88), where the observed eating time was 259.2 min/day, rumination time 343 min/day, milk yield 26.21 kg/day and milk fat content 4.11%. An increased chewing time was achieved at lower value of THI, as well as higher milk yield and improved milk composition.

Keywords: dairy cattle, production performances, chewing activity, THI

Introduction

Heat stress is one of the major external factors that can negatively affect the performance of dairy cows (Nardone et al., 2010). This finding is especially true in high-yielding animals of high genetic potential which are very sensitive to heat stress (Bernabucci et al., 2014). Several studies hypothezised the 'thermo-neutral zone' (i.e. the thermal equilibrium between the animal and the environment for lactating dairy cows to be between 5°C and 25°C to 26°C (Berman et al., 1985). Whenever the temperature exceeds this thermal zone,

[1] University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Zemun, Serbia (blasko.stojkovic@gmail.com)

trespassing either the upper or lower limit, animal physiology changes to a disorder status in which, for example, milk production declines and its composition changes (Johnson, 1980).

Several studies have already highlighted the negative relationship between THI and productive and reproductive performances in dairy cows (Bernabucci et al., 2014, Biffani et al., 2016), but few studies investigated the relationship between THI and rumination time. Lactating cows spend about 4.5 hours/day eating (range: 2.4–8.5 hours/day) and 7 hours/day ruminating (range: 2.5–10.5 hours/day), with a maximum total chewing time of 16 hours/day (Beauchemin, 2018). Peak chewing activity during consumption usually occurs after meal distribution (King et al., 2016) or during the subsequent redistribution of bulk portions of the meal during the day. Bar and Solomon (2010) reported that imbalances in animal welfare, such as heat stress, could be detected by a decrease in rumination time. Rumination time is positively correlated with milk yield (Antanaitis et al., 2018). Acatincai et al. (2009) concluded that when the temperature exceeds the upper limit of thermal comfort of a particular breed, the rumination process is severely affected. Temperatures beyond 27–28 °C reduce the overall rumination process, including the frequency and duration of this activity. Muschner-Siemens et al. (2020) observed that rumination time is affected by several individual cow factors even in moderate climates, indicating that the parameter could be used to evaluate the effect of climate change on dairy cows. Abeni and Galli (2017) defined a value of 420 min/day as critical for rumination time in dairy cows.

The recent introduction of an indirect method to measure rumination time, based on the analysis of vocal signs (HR-Tag rumination monitoring system), allows automatic measurement of rumination time and analysis of rumination behavior during the daytime and nighttime (Soriani et al., 2013).

The purpose of the conducted experiment was to determine how the different values of THI affect eating time and rumination time, as well as the milk yield and chemical composition of milk.

Material and Methods

Data were collected from 70 Simmental dairy cows, reared in the farm "DMN" Malo Crniće, Požarevac.

The research included determining the effects of different values of surrounding temperature and humidity on eating and rumination time, as well as on the milk yield and chemical composition. The research consisted of two

experimental parts of 42 days each in different periods of the year (autumn/summer). During both experimental periods, for lactating cows' nutrition, an identical PMR (Partially Mixed Ration) was used. The number of cows in the group was 35, with an average age of 40 months and an average of 170 days in milk (DIM). For both groups of cows, based on the average milk yield, a ration was formulated for the production of 29 kg of milk with 3.6% milk fat and 3.2% protein. The partial mixed rations (PMR) used were uniform in terms of chemical composition for both experimental groups of cows. The composition and nutritional value of the partial mixed ration are given in Table 1. During the experimental periods, the partial mixed rations for lactating cows consisted of identical feeds.

Table 1. The composition and nutritional value of PMR

Item	Partially mixed ration, kg
Alfalfa hay	2.64
Corn silage	8.25
Straw	0.44
Concentrate 21,6% CP	7.83
Dry matter, DM, kg	19.16
In DM of ration	
Crude protein, %	15.31
Neutral detergent fibers, %	34.42
Acid detergent fibers, %	21.20
Starch, %	19.85
Ca, %	0.83
P, %	0.30
NEL, MJ kg^{-1}	6.71

In addition to PMR, both groups of cows received 3 kg of concentrate with 12% CP during milking (3 times per 1 kg of concentrate).

The trailer mixer feeders - BVL with a volume of 8 m^3 were used for the preparation and distribution of ration. The ration was distributed to animals once a day at 8 am.

Ambient temperature and relative humidity were recorded using the Testo 174H data logger istalled inside the stall. Both ambient temperature and relative humidity were measured every hour. The guaranteed working range, as

indicated in the manufacturer manual, was from - 20°C to 70°C for the ambient temperature and from 0% to 100% for the relative humidity.

According to National Research Council [NRC] (1971), the THI can be written in the following way, where T and H are the average daily temperature (Celsius scale) and average relative humidity (%):

$$THI = (1.8\times T+32) - [(0.55-0.0055\times H)\times(1.8\times T-26.8)]$$

All cows participating in the experiment were equipped with GEA CowScout Neck sensor collars that monitored eating time and rumination time, which were registered using GEA DairyRobot R9500 software.

Milk yield was recorded daily, while milk fat and protein content were determined at 15-day intervals.

To analyze the statistical significance of the differences for the determined values of the observed parameters - THI, eating time, rumination time, milk yield and chemical composition, the Student's t-test was used, at the level of significance $p<0.01$ and $p<0.05$. The parameters of descriptive statistics and standard deviation were also determined.

Results and Discussion

Considering the presence of heat stress, the obtained results of the THI measurement, indicated that during the autumn period, cows were in the safe zone, while the discomfort zone characterized the summer period. The determined THI values are given in Table 2.

Table 2. The THI values in the experimental periods

Week of the experimental period	Autumn period	Summer period	Significance
I	58.58 ±5.27	71.42±5.36	**
II	49.25±5.36	75.30±4.80	**
III	54.91±7.02	70.59±4.57	**
IV	50.56±7.78	74.89±5.99	**
V	54.94±5.71	71.69±5.37	**
VI	47.09±5.41	73.38±6.01	**
Average THI	52.55 ± 5.59	72.88 ± 5.35	**

± - standard deviation ; ** - $p<0.01$

According to Hahn et al. (2003), six THI thresholds for heat stress classification were used, namely 'safe' (THI<68), 'mild discomfort' (68⩽THI<72), 'discomfort' (72⩽THI<75), 'alert' (75⩽THI<79), 'danger' (79⩽THI<84), and 'emergency' (THI⩾84). The THI is also categorized according to Zimbelman and Collier (2011), as follows: THI < 68 as no stress, 68≤ THI < 72 as mild stress, 72 ≤ THI < 80 as moderate stress, and THI≥ 80 as severe stress. Kadzere et al. (2002) reported that heat stress is beginning in subtropical regions with a THI value of 70. On the other hand, Schüller et al. (2014) described that heat stress is already developed at low THI units in moderate climates.

The significant differences in THI values for observed periods had an effect on eating time and rumination time, which were shown in Table 3.

Table 3. Eating time and rumination time in cows for different THI values

Chewing activity	THI_1	THI_2	Significance
Eating time (min/day)	307.2±*21.6*	259.2±*31.08*	**
Rumination time (min/day)	384±*25.2*	343±*27.0*	**

THI_1 - 52.55; THI_2 - 72.88; ± - standard deviation; ** - p<0.01

Statistical analysis of the data revealed that the eating time of the observed groups of cows differs significantly, which can also be concluded for the rumination time. The obtained values for chewing activity during rumination in lactating cows are lower compared to the results reported by Bar and Solomon (2010), where the average rumination time during lactation was determined to be 478 min/day. In a study involving 515 dairy cows in 7 experiments, the cows consumed an average of 7.7 meals per day, with a time interval between meals of at least 29 minutes (De Mol et al., 2016).

Moallem et al. (2010) indicated that the primary negative effect of high THI is a depression of rumination time, which subsequently led to a reduction of dry matter intake, followed by a decline in milk yield. When temperature exceeds the threshold values, a 1°C increase in daily mean temperature reduced rumination time by 5.12 min per day and reduced rumination efficiency by 0.07 kg DM per cow per hour (Ji et al., 2020). In another study, total rumination time, day rumination and night rumination time were reduced with the high temperatures in early, mid and late lactation in dairy cows (Abeni and Galli, 2017). More interestingly, heat stress reduced rumination time by 22.9% even in dried-off dairy cows, leading to reduced degradability of the feed

consumed (Maia et al., 2020). Soriani et al. (2013) found a significant negative correlation (r=−0.32) between THI and rumination time.

Different values of THI had an impact on the milk yield as well as on the chemical composition of milk (Table 4.)

Table 4. Impact of different THI values on milk yield and composition

Ithem	THI_1	THI_2	Significance
Milk yield, kg/day	28.97±2.22	26.21±2.46	**
Milk fat content, %	4.25±0.02	4.11±0.04	*
Milk protein content, %	3.80±0.04	3.81±0.03	ns

THI_1 - 52.55; HI_2 - 72.88; ± - standard deviation; ** - $p<0.01$; *- $p<0.05$; ns - not significant

By comparing the data, it was determined that the milk yield of the two observed groups of cows is significantly different. Daily milk yield in the group of cows in the period of the year with the lower value of THI was higher ($p<0.01$) compared to the group of cows in the period of the year with the higher value of THI. Also, content of milk fat in this group of cows was significantly different ($p<0.05$). The milk protein content was not statistically different in the observed groups.

Internal metabolic heat production during lactation can reduce the resistance of cattle to high ambient temperature, resulting in altered milk composition and reduction in milk yield (Hossein-Zadeh et al., 2013). Also, Rajeb et al. (2012) determined that heat stress affects the reduction of fat and protein in milk.

Heat stress involves hormonal and metabolic changes, as anabolic hormones decline and catabolic hormones increase, which alter energy balance. High temperature stress affects production and reproduction performance by decreasing antioxidant enzyme activity, which increases oxidative damage in the tissues, and by changing carbohydrate, lipid, and protein metabolism (Soriani et al., 2013).

Conclusion

The lactating dairy cows used in this study suffered mild to moderate heat stress in some periods of the trial. Chewing activity during consumption and rumination is necessary from the perspective of ensuring normal function of the rumen, efficient utilization of consumed food and optimal production. Based on

the results of this experiment, it can be concluded that different values of THI affect eating time and rumination time, as well as milk yield and milk fat content. THI of 52.55 was found to have a beneficial effect on chewing activity, milk yield and milk fat content compared to THI of 72.88. In order to achieve optimal production results, it is necessary to pay more attention on factors affecting the ambient temperature and relative humidity on dairy farms. Also, in periods with higher THI values, it is necessary to adjust the composition of diet and the feeding technology of productive animals. These results support the use of rumination and eating time as a heat stress markers, whose measurement during the summer season provides useful information on the potential levels of heat stress that often affect cows. Further research is necessary to verify whether the primary negative effect of high THI is the depression of rumination and eating time, which subsequently leads to a reduction of dry matter intake.

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THE INFLUENCE OF INOCULATION ON THE CHANGE OF NITROGEN SUBSTANCES IN SILAGE

Blagoje Stojković[1], Nenad Đorđević[1], Aleksa Božičković[1], Saša Obradović[2]

Abstract: The paper presents a comparative overview of the influence of homofermentative and heterofermentative lactic acid bacteria (LAB) on the degree of proteolysis and aerobic stability in Fabacea and Poacea silages. When using Lactobacillus buchneri, compared to Lactobacillus plantarum, in alfalfa silages, a significant increase in the pH value (4.67:4.23), a decrease in the amount of lactic acid (11.8:24.3 g/kg DM) and an increase in the amount NH3-N (70.6:49.8 g/kg N). When using heterofermentative LAB (L. hilgardii and L. Buchneri) compared to the control treatment, a significant increase in the acetate:lactate ratio (3.64:1.85) and a decrease in the number of yeasts was found in corn silages, while there were no significant differences in pH value and amount of NH3-N. Due to the often negative impact of heterofermentative LAB on proteins in leguminous silages, research should be continued in order to determine the best LAB combinations for corn silage, where probiotic yeasts should also be included.

Key words: inoculation, LAB, silage, proteolysis, probiotic.

Introduction

The use of biological additives (inoculants) based on lactic acid bacteria has been current since the beginning of the last century (McDonald et al., 1991). Compared to other supplements tested and/or used in practice (mineral and organic acids, their salts...), the advantage of biological supplements is that they do not leave residues and do not negatively affect the health of animals and the quality of their products (Đorđević et al., 1999, 2001; Ogunade et al., 2017). Because, they are also allowed in Serbia by the relevant regulations on organic production in agriculture (Đorđević et al., 2014). However, the disadvantage of this type of additives is primarily in the smaller effect on the pH value of the silage, which is one of the main factors for controlling the degradation of nutrients. The use of selected strains of homofermentative lactic acid bacteria

[1] University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Zemun, Serbia

[2] University of Niš, Faculty of Agriculture in Kruševac, Kosančićeva 4, 37000 Kruševac, Serbia

(LAB) intensifies and directs fermentation, primarily in feeds that do not contain enough fermentable carbohydrates (leuminose), while the "II and III generation" of inoculants also contain heterofermentative LAB, in order to increase aerobic stability and longer useful values of mycotoxin-free silage (Addah et al., 2012; Blajman et al., 2018; Ferrero et al., 2019; Muck et al., 2018). Some heterofermentative LAB (for example, Lactobacillus buchneri) also create acetic acid in anaerobic conditions as a strong fungicidal agent, which protects silage in contact with air. A problem that is still very relevant is the degree of changes in nutrients, primarily proteins, when LAB supplements are used, especially when comparing the efficiency of homofermentative and heterofermentative LAB in silages from the same or different plant material.

Types and volume of protein changes upon inoculation

Changes in nitrogenous substances, which are significant for production results, health and reproduction of ruminants, are particularly characteristic of legumes silages (Đorđević et al., 2010, 2011a, 2012). Hydrolytic decomposition of proteins under the action of plant cell enzymes or microorganisms has a negative effect on the utilization of these specific building materials due to an increase in the fraction of degradable proteins in the meal (Đorđević et al., 2011b, 2018, 2022). The basic indicator of protein degradation in silage is the amount of ammonia nitrogen, which is relatively easily (and cheaply) detected in laboratories (Đorđević et al., 2004). However, modern standards require more detailed and precise knowledge of fractions of nitrogenous substances in the meal (NRC, 2021), which requires the use of more expensive equipment and instrumental methods.

The influence of inoculation on the pH value, and thus on the degree of changes in nitrogen substances in the silage, is usually significantly higher compared to the control treatment. However, even in experiments where no significant difference was found in the proportion of ammonia nitrogen during silage inoculation, a significantly lower content of butyric acid (also a product of proteolytic clostridia) was observed, due to the absolute and relative increase in the proportion of lactic acid, as a typical bactericidal agent (Liu et al., 2018).

The use of inoculants has so far been mostly tested on legumes, where they were used with the aim of intensifying fermentation and rational consumption of non-structural carbohydrates, the quantities of which are insufficient for self-preservation. Therefore, the best results were achieved when using homofermentative LAB. Liu et al. (2018) used three types of supplements when

ensiling alfalfa: an inoculant with the "classic" (homofermentative) lactic acid bacterium L. Plantarum (due to the bactericidal properties of lactic acid and the effect on the pH value), an inoculant with the heterofermentative lactic acid bacterium L. Buchneri (in in order to increase the aerobic stability of silage) as well as propionic acid (due to its fungicidal properties). The significantly lowest pH values were found in the treatments with L. Plantarum (4.23) and propionic acid (4.41), while the highest amount of lactic acid was found in the treatment with L. Plantarum (table 1). Except in the control silage, the highest amount of acetic acid was determined in the treatment with L. Buchneri, although this was not a significant difference compared to the treatment with L. Plantarum. The amount of ammonia nitrogen was the lowest in the treatments with L. Plantarum and propionic acid, which once again confirms the fact that the pH value of the silage is of key importance for the degree of proteolysis. In addition, these treatments had the significantly lowest butyric acid content, also due to the direct effect of pH value, but also the bactericidal effect of lactic acid. After 6 days of exposure to oxygen, there was no statistical difference in the number of yeasts (responsible for secondary fermentation), between treatments with L. Buchneri and L. Plantarum. That fact, as well as the fact that the treatment with L. Buchneri had the highest degree of proteolysis expressed through the proportion of ammonia nitrogen, raises the question of the justification of using heterofermentative LAB in ensiling legumes.

Table 1. Effect of additives on the fermentative characteristics of silages (Liu et al., 2018)

Items	pH	Lactic acid	Acetic acid	Butyric acid	NH_3-N g/kg N	Yeasts Lgcfu/g FM
		g/kg DM				
Control	5.43a	0.59c	32.5a	32.7a	155a	2.90
LB	4.67b	11.8b	25.5ab	12.9b	70.6c	<2.00
LP	4.23c	24.3a	20.27ab	1.43c	49.8d	<2.00
PA	4.41a	10.9b	15.7b	0.73c	47.3d	<2.00
TP	5.30a	0.36c	32.6a	31.5a	129b	<2.00
P-value	<0.001	<0.001	0.038	<0.001	<0.001	0.208

Values with different letters (a-d) in the same row indicated a significant difference according to Tukey's test ($p<0.05$); LB: *Lactobaccilus Buchneri*; LP: *Lactobaccilus Plantarum*; PA: Propionic acid; TP: tea polyphenols.

Nair et al. (2020) examined the results of using two types of homofermentative LAB in corn silage: L. hilgardii and L. Buchneri. Comparing

the control and inoculated silage, the authors determined a significant increase in the proportion of acetate (13.9:26.2 mg g−1 DM), and a decrease in the ratio of lactate and acetate (3.64:1.85), but there were no significant differences in pH values and amount of ammonia nitrogen. Also, the authors found a significant decrease in the number of yeasts (4.32:0.91 log10 cfu g−1 DM), which achieves greater aerobic stability of the inoculated silage. In experiments with animals, no significant differences were found in terms of digestibility and production performance. The authors believe that the most important benefits when using heterofermentative LAB inoculants are the lower intensity of silage compaction and the opening of a larger front of the used silage.

Xu et al. (2019) examined the results of using the "fourth" generation of inoculants, which in addition to L. Buchneri also contained the yeast Saccharomyces cerevisiae. It was found that S. Cerevisiae did not bad affect the quality and aerobic stability of the silage despite the increase in numbers after opening the silage, while L. buchneri did not prevent spoilage as the pH in all silages was on average 8.0 after 7 days of aerobic exposure. In earlier experiments, it was found that silages supplemented with S. Cerevisiae showed higher levels of nutrient utilization and lower methane emissions (McAllister et al., 2011). It is obvious that modern inoculants containing LAB and yeasts do not negatively affect silage quality and use value and may have probiotic properties.

Conclusion

When using individual LAB species in Fabaceae and Poaceae ensiling experiments, the most precise data on their influence on changes in the amount of fermentation products and aerobic stability of silage are obtained. However, in practice, different combinations (mixtures) of LAB microorganisms are used, due to which mutual interactions occur and completely new results are obtained. The significant influence of heterofermentative LAB on proteolysis in leguminous silages, where the problem of aerobic stability is less pronounced in practice, has been experimentally proven. That is why the use of heterofermentative LAB in the ensiling of buttercups is quite debatable. In any case, research on the effectiveness of commercial inoculants containing both groups of LAB, as well as yeasts with the role of probiotics, should be continued in order to determine the best combinations.

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ECONOMIC RESULTS OF BROILERS PRODUCTION ON THE FAMILY FARM

Biljana Veljković, Milica Kostić, Simeon Rakonjac, Ranko Koprivica, Marija Gavrilović, Milun Petrović[1]

Abstract: The fattening of broilers in Serbia is partially organized through contract production in small family farms that fatten broilers for the needs of large companies. The article contains an economic analysis of this small family farm, which produces about 12 000 kg of chicken meat per year on a small area of 120 m^2, with one family member involved all the time and other members helping as needed. Fattening broilers on the farm is organized in two ways: contract fattening up to 1 kg for 25 days and fattening up to 3.5-4 kg for 56 days. In the case of fattening broilers up to 25 days of age, on average, feed costs account for 45%, day-old chicks for 26%, and labor costs for 22%. For broilers up to 56 days of age, the largest average share is feed costs 62.4% and labor costs 26.2%. The price of fattened broilers did not change during the fattening period, so the realized production value was the same in one fattening method and similar in the other fattening method, while cost of production increased in each fattening round, which affected the reduction of contribution margine. In addition to the increase prices of feed mixtures, positive economic results were achieved on the farm, and with contract production, secure purchasing was ensured and risks in production were reduced.

Keywords: broiler chickens, costs, contribution margin

Introduction

Compared to other agricultural production, broiler production time is short, so the turnover and return on invested funds are faster. At the same time, the feed conversion ratio is good, as well as the growth of broilers. In the production of chicken meat, large-scale producers have become much more competitive in the market through intensification and modernization, so many farms have abandoned this production. In order for farms to survive in this production, they must be market-oriented or be part of the contract production for larger producers. To maintain the domestic chain of chicken meat production, family farms can supply catering fascilities through agritourism

[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia (biljavz@kg.ac.rs)

and be part of the organic production program. Such small businesses on family farm linked to market contribute significantly to rural development.

In Serbia, 13 kg of chicken meat is produced per inhabitant, and consumption is 17 kg per inhabitant per year (Vlahović, 2015). The main reason for importing chicken meat is the lower price of imported chicken meat. In foreign trade of poultry meat, Serbia achieves a negative balance of 7.7 million USD in 2021. Whereby this deficit is even more pronounced in 2022, amounting to 16.7 million USD, according to the official data of Statistical office of the Republic of Serbia. The largest imports of frozen chicken meat come from the EU, Russia and CEFTA group countries, with the trend continuing to increase.

Farms with smaller production capacities should focus on fattening high-quality broilers, use quality feed, and have good health protection conditions. Regarding genetic potential, selection of broilers with fattening traits, faster growth and more efficient feed conversion has made significant progress. Today's broilers differ significantly from past broilers in terms of body weight, feed conversion, and carcass characteristics (Kokoszynski et al., 2017; Hurcher and Lum, 2020; Torrey et al., 2021).

For an economic production of broilers, it is necessary to match the capacities of the farm and their utilization. This is because housing conditions and stocking density per m^2 for broiler significantly influence production results (Mitrović et al., 2010; Salihbašić et al., 2014, Weimer et al., 2020).

It is very important that broiler chickens at a certain stage of fattening receive an adequate amount of energy, proteins, minerals and vitamins, as this ensures intensive growth and development of the broiler. With a carefully balanced meal and proper selection of hybrids for fattening under controlled conditions, good production results are achieved (Dosković, 2015).

The duration of a run in broiler fattening is relatively short, which is why Subić et al. (2010) and Jeločnik et al. (2021) recommend that farms can influence the increase in farm results at the calendar year by increasing number of fattening cycles or the number of chicken in turn of fattening.

Materials and methods

The study was carried out on a family farm, which has many years of experience in fattening chickens and is located near Vrnjačka Banja. The capacity of the poultry farm for fattening is 1000 chickens up to 1 kg and 650 chickens up to 3.5-4 kg. For 7 years lasts, the contract production of service fattening of broilers Cobb500 up to 1 kg has been running with a large

company, with which the farm successfully cooperates. The broilers weighing 3.5-4 kg are fattened on the farm for 56 days and are intended for regular customers. The farm strives to meet the needs of its customers and organize more cycles of fattening during the year. The family farm with an area of 10 hectares also grows forage crops, which is in the function of livestock, other types of agricultural production are also represented, and broiler chicken fattening is only one of them. One member of the household is constantly engaged in fattening the chickens, and the other members help when necessary.

In this family farm, chicken fattening was investigated in 2021. The study included both methods of fattening with three cycles of fattening each. The organizational and technological processes in production were monitored, costs were recorded, and calculations were made. The economic profitability of production was determined using the method of calculating the coverage of variable costs. That is calculating the contribution margine as the difference between the realized production and the amount of variable costs (Subić et al., 2010; Jeločnik et al., 2021). The input prices for each fattening cycle were recorded, and were given calculations of the contrabution margine in which the realized economic results were calculated.

Results and discussion

The floor system for broilers production is carried out in facilities with adequate insulation and partially mechanized equipment, with part of the work done manually. Chickens are reared on a deep litter with manually refilled feeders that hold 11 kg of feed and are adjusted to the age of the birds during production. The waterers are partially mechanized and are refilled daily with fresh water to which vitamins and medicines are added according to technological requirements. The combination of ventilation and heating, as well as the control of the drinkers, maintains the required humidity in the room at about 70%. Depending on the months in which fattening is carried out, the ventilation and heating of the poultry house is adjusted. During each cycle of fattening, day-old chicks are housed in a clean, disinfected and well-heated room with a dry litter. Day-old chicks are very sensitive and must be provided with the required temperature and appropriate conditions. During fattening, the condition and behavior of the chickens are monitored to produce healthy individuals.

In broiler feeding, feed mixtures are used, i.e. Starter, Grover I and Grover II. In performance fattening according to the technology of controlled fattening,

feed mixtures are purchased from the company, namely initial feed with 22% crude protein and full feed with 19% crude protein. For longer fattening tours, feed mixtures with 17% crude protein are used. For conversion of feed for fattening up to 1 kg, 1.5 kg of feed is consumed. For fattening broilers with an average weight of 3.5-4 kg weight, 7 kg of feed is consumed. After three weeks of fattening broilers reach a weight of 1 kg. Service fattening of 1000 broilers is completed, the company picks them up and brings them to the desired place. On the farm remain 650 broilers and are fattened for up to 56 days. When they reach the desired weight, they are slaughtered, cleaned, washed, packed and delivered to customers.

The calculation of costs and contribution margins for the service production of fattening up to 1 kg in all three cycles is shown in Table 1. The contracted price for one broiler chicken was 220 dinars or 1.86 EUR and did not change in all three cycles of fattening. In the calculation of income, manure is added as a by-product, and on average about 2 tons are obtained per round. Due to the low capacity of broiler fattening, the farm does not receive subsidies for this production. During the observed period, costs for all three fattening cycles increased by 11%, energy costs were in low growth, which affected the reduction of contribution margine, which was the lowest in the third fattening pass, 340 EUR. The average share of feed costs for all three cycles of fattening is 45.3%, and these are also the largest costs. Day-old chick costs are represented with an average share of 26%, labor costs with 22%, and followed by other costs with a lower share (Table 1).

Table 1. Calculations of the fattening cycle of broilers up to 1 kg for 25 days

Production value	**Fattening I**	**Fattening II**	**III Fattening**	**Schare in %**
Fattened broilers	1865	1865	1865	97.8
Manure	42	42	42	2.2
Total income	**1907**	**1907**	**1907**	**100**
Costs				
One-day chicks	398.3	398.3	398.3	26
Feed	654.2	703.4	728.2	45.3
Medicines	25.4	25.4	25.4	1.7
Energy sources	29.7	33.9	33.9	2.2
Straw and others cost	16.9	16.9	16.9	1.1
Veterinary services	16.9	33.9	25.4	1.7
Labor costs	339	339	339	22
Total costs	**1481**	**1551**	**1567**	**100**
Contribution margin	**426**	**356**	**340**	**/**

Labor costs were calculated for one worker employed per day during the 25 days of the fattening shift. The average variable cost in the total calculation was 1.53 EUR per broiler. Contract fattening with a large company eliminates for the farm the cost of transporting day-old chicks, feed mixture and fattened broilers, as well as the risk of repurchase, i.e. sale.

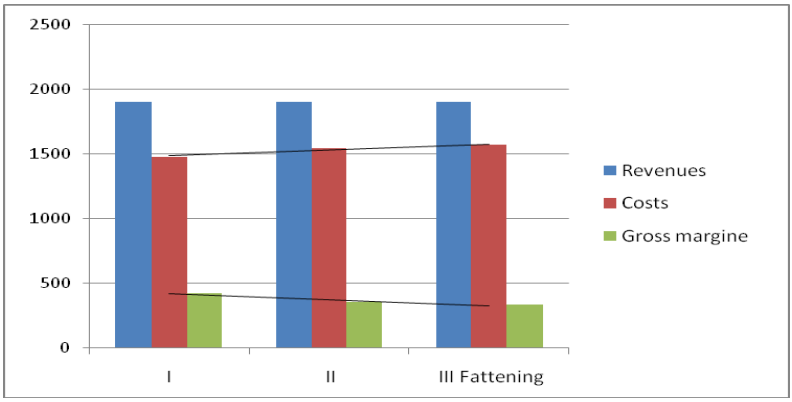
The calculation of cost and contribution margin of the extended fattening of 56 days for own customers for all three runs is shown in Table 2. The selling price of fattened broilers has not changed in all three fattening passes and is 280 dinars per kg or 2.37 EUR. Approximately 3 tons of manure are produced per fattening cycle, the value of which is included in the income. Due to the increase in feed prices with each new fattening cycle, feed costs increased. More feed was used in this type of fattening, so the average share of feed costs was 62.4%. Labor costs had an average share of 26.2% and were calculated for a worker employed for 68 days in one fattening cycle. The lowest contribution margin of 1 734 EUR was obtained in the fattening round III (Table 2). The average variable cost in the total calculation was 5.53 EUR per broiler.

Table 2. Calculations of the fattening cycle of broilers up to 3.5-4 kg for 56 days

Production value	**Fattening I**	**Fattening II**	**III Fattening**	**Schare in %**
Fattened broilers	5382	5363	5369	98.8
Manure	64	64	64	1.2
Total income	**5446**	**5427**	**5433**	**100**
Costs				
One-day chicks	259	259	259	7.4
Feed	2059	2169	2366	62.4
Medicines	25.4	25.4	25.4	0.7
Energy sources	76.3	67.8	84.7	2.2
Straw and others cost	25.4	16.9	25.4	0.6
Veterinary services	25.4	16.9	16.9	0.5
Labor costs	922	922	922	26.2
Total costs	**3392**	**3477**	**3699**	**100**
Contribution margin	**2054**	**1950**	**1734**	/

Both calculations showed that food costs have a large share in variable costs, which is in line with studies by other authors (Veljković, 2015; Horne, P.L.M. van, 2018)

The interdependence of the obtained economic results from the calculations for the first fattening method is shown in Graph 1.



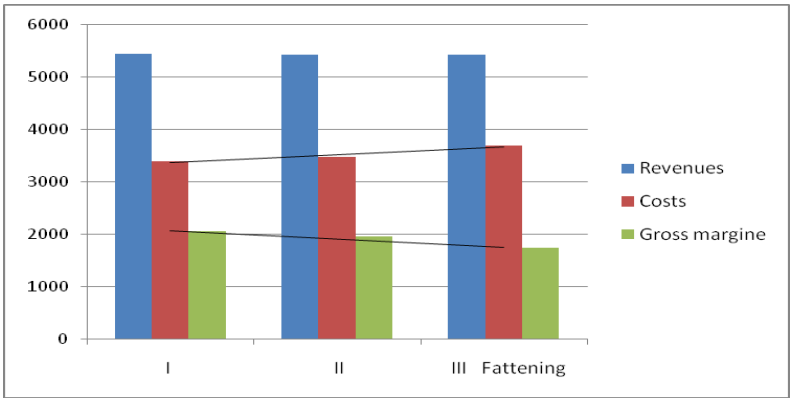
Graph 1. Economic results of realized fattening up to 1 kg for 25 days

The incomes in all three fattening cycles did not change, and the contract price for fattened broilers was the same. The increase in feed prices also increased the cost of production, resulting in a 21% decrease in the contribution margin in the fattening round III. In the first fattening method, the realized contribution margin in relation to the realized production value is on average 19.5%.

A comparative overview of the economic results obtained for the other type of fattening is shown in Graph 2. The incomes in the fattening cycles showed slight variations, considering that the weight of the fattened broilers varied between 3.5 and 4 kg, while the prices of the live weight of the broilers were the same. Feed prices increased during fattening and the contribution margin decreased by 16% in the round III. In terms of realized production value, the contribution margin averaged 35.1%.

The decrease in contribution margin in both fattening methods was due to an increase in variable costs, especially feed costs. The contribution margin is sensitive to the increase in prices for concentrate mixtures, and if broiler feed prices continue to increase, production would no longer be economically viable.

The contribution margin should also cover fixed costs, which are not included in these calculations. In the studies by Subić et al. (2010) and Jeločnik et al. (2021), it was shown that the contribution margin is extraordinarily sensitive to changes in fatten broiler prices and if be eventual decrease would lead to negative economic results.



Graph 2. Economic results of realized fattening up to 3.5-4 kg in duration of 56 days

From the comparison of these two types of fattening, it can be concluded:

- In the first type of fattening, the obtained contribution margins are lower, but the fattening takes short time and the risks are minimal. The observed family farm takes advantage of all the benefits it can obtain from contract production.

- In the second type of fattening, the investments are higher, the contribution margins are higher, the fattening lasts longer and there are greater risks in selling and placements.

The decision on the profitability of production is made on the farm itself, taking into account all the production parameters presented. So, the farm should weigh and make the best decision on what is more profitable for them. In the case of this study, both fattening methods were combined.

Conclusion

The agricultural sector of the European Union has been developing for many years under the conditions of a stable agricultural policy and with high state subsidies. In Serbia with agricultural production in small farms solves the current social problems and employs at least one family member, which are short-term solutions that are hardly comparable. Under these conditions, domestic production is less and less competitive on the market. Because of their low capacity, family farms are not recognized for receiving government incentives and subsidies, so they must seek this type of support under rural development. Farmers of fattening broilers also face such problems.

For this reason, some agricultural producers in search of alternatives in production develop family business.Thus, in addition to tradition and inherited way of life, agriculture is increasingly becoming a financially profitable business. These family farms orient themselves to the market, find steady buyers for their products and conclude production contracts with business partners.

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EFFECT OF PROTEASE ADDED IN FOOD AND SEX ON CHICKEN MEAT CLASESS

Vladimir Dosković[1], Snežana Bogosavljević-Bošković[1], Zdenka Škrbić[2], Božidar Milošević[3], Miloš Lukić[2], Simeon Rakonjac[1], Veselin Petričević[2]

Abstract: The effects of feeding low dietary crude protein with supplemental protease and sex on the weight and percent yields of individual meat classes in broiler chickens of hybrid Cobb 500 were investigated. A total of 300 day-old broiler chicks were fed with one of the following three experimental diets: control group (C), the experimental group I (E-I) contained 4% less crude protein than the control (C) and were supplemented with protease (Ronozyme Pro Act) at a concentration of 200mg/kg feed and the experimental group II (E-II) contained 6% less crude protein and were supplemented with protease (Ronozyme Pro Act) at a concentration of 300mg/kg feed.

Supplementation of protease to diets had no significant effects on weights of individual meat classes (P>0.05), as well as the percentage of meat classes, and the only differences were manifested between E-I and E-II (P<0.05, female broilers from the E-I group were had a higher percentage of class I meat and a lower percentage of class III meat compared to females from the E-II group). The effect of sex was manifested in the weights of all meat classes and the percentage of class I meat and class III meat (P<0.05).

Keywords: fattening chickens, meat classes, protease, sex

Introduction

Protein is the second major nutrient (after energy) and the most expensive in the broiler diet. The protein sources in modern poultry diets are mostly derived from soybean products (Dosković et al., 2020).

Lately, the use of proteases as feed enzymes has gained interest. Proteases are added to feed to increase dietary protein hydrolysis and thus enabling improved nitrogen utilization. When animals utilize nitrogen better, there is a

[1]University of Kragujevac, Faculty of Agronomy Čačak, Cara Dušana 34, Čačak, Serbia
*Corresponding author: vladosko@kg.ac.rs
[2]Institute for Animal Husbandry, Autoput 16, Poštanski fah 23, 11 080 Belgrade-Zemun, Serbia
[3]Faculty of Agriculture, University of Pristina, Kosovska Mitrovica-Zubin Potok-Lešak, 38219 Lešak, Serbia

possibility to decrease the diet protein content and in turn also reduce the content of nitrogen in manure (Oxenboll et al., 2011). The use of protease as a feed additive offers significant environmental benefits: it decreases the pollution of water and air with nitrous compounds leading to eutrophication and acidification, decreases the health risks associated with NH_3 emissions in broiler houses and can contribute significantly to reducing nitrogen emissions from livestock production.

Dietary supplementation of protease to low crude protein and/or metabolisable energy diets showed negligible effects on growth performance, carcase characteristics and physiological responses in broiler chickens under heat stress conditions. The inclusion of microbial protease in broiler diets could be considered by the poultry industry as an effective nutritional tool for reducing metabolisable energy or crude protein, to decrease abdominal fat deposition, improve feed efficiency and increase the profit margin (Law et al., 2019).

Many researchers have investigated the effects of protease and reduction of crude protein levels on carcass characteristics and meat quality of broilers (Mahmood et al., 2017; Park and Kim 2018; Law et al., 2019; Dosković et al., 2020; Dosković et al., 2022;…).

The hypothesis was that protease supplementation would increase the digestibility of crude protein and amino acids in diets that reduced crude protein (groups E-I and E-II) and would lead to the same carcase quality as the standard diet (group C).

Materials and methods

A total of 300 one-day-old Cobb 500 broiler chickens males and females were randomly divided into 3 experimental units of 100 chickens each: C - control group, standard diet, without protease enzyme; E-I group - 200 mg Ronozyme ProAct/kg feed and 4% less crude protein than the control group and E-II group - 300mg/kg feed and 6% less crude protein than the control respectively.

The fattening period was divided into 3 phases: starter (1 to 21 d) and grower (22 to 35 d) and finisher (36 to 49 d). Feed (mash form) and water were provided *ad libitum* throughout the experimental period.

At the end of the experiment, at 49 day of age, 20 chickens per treatment (10 males and 10 females) were randomly selected for carcass quality measurement.

The selected chickens after slaughter, were dissected into primal cuts (according to the Commission Regulation (EC) No. 543/2008) and divided into meat classes (class I meat: breast, drumsticks and thighs; class II meat: wings and class III meat - back and pelvis), and then the weights of individual meat classes were measured. Based on the weights of the meat class and ready-to-grill carcass weights, the proportions of the meat class in the dressed carcass were determined.

The results were analyzed by Stat Soft Inc Statistica For Windows (Version 7.0., 2006) program using the two-factor analysis of variation (diet treatments and sex). Data were followed by the Tukey test (P<0.05).

Results and discussion

Data on the weight of meat classes, by feeding treatments and sexes, are presented in Table 1.

Table 1. Weight of different classes of chicken meat on the 49th day of fattening, gr

Treatment			Class I (breast, thighs, drumsticks)	Class II (wings)	Class III (back, pelvis)
Groups	Sex				
C	Male	$\bar{X}$	1630.47[a]	285.43[a]	577.61[a]
		Sd	72.86	15.49	32.00
	Female	$\bar{X}$	1373.74[b]	238.33[b]	481.88[b]
		Sd	93.36	9.57	35.71
E-I	Male	$\bar{X}$	1544.84[a]	275.06[a]	586.46[a]
		Sd	99.46	11.96	32.37
	Female	$\bar{X}$	1407.61[b]	229.24[b]	453.03[b]
		Sd	91.71	15.58	40.60
E-II	Male	$\bar{X}$	1561.07[a]	277.90[a]	583.68[a]
		Sd	104.18	20.37	64.29
	Female	$\bar{X}$	1306.81[b]	229.77[b]	480.67[b]
		Sd	35.94	14.81	32.50

$\bar{X}$-Average, Sd - Standard deviation
Different superscripts (a, b) indicate a statistical significant differences between groups (P <0.05)

Analysis of the data in Table 1. showed that no significant differences were observed in the weight of all class meat between dietary treatments (P>0.05). Similar results, that the mass of certain parts of the carcass and meat classes has no effect on reducing the content of raw proteins, with the addition of protease enzymes are indicated by Jawad (2017), Dosković et al. (2020), Matshogo et al.

(2021),… Male chickens had a larger mass before slaughter compared to female chickens, as well as a larger ready-to-grill carcass weights, which was reflected in the mass of all meat classes (class I meat: breast, drumsticks and thighs; class II meat: wings, and class III meat back and pelvis) (P<0.05). Brewer et al. (2012), Dosković et al. (2020) and Kamporn et al. (2022) state that the sexes of the chickens have a significant influence on the weight of the primal carcass cuts, which make up the meat classes.

Table 2. The percentage of different classes of chicken meat in the dressed carcass on the 49th day of fattening, %

Treatment			Class I (breast, thighs, drumsticks)	Class II (wings)	Class III (back, pelvis)
Groups	Sex				
C	Male	$\bar{X}$	63.14^{ab}	11.06	22.38^{ab}
		Sd	1.45	0.53	1.04
	Female	$\bar{X}$	63.11^{ab}	10.97	22.14^{ab}
		Sd	1.25	0.55	0.98
E-I	Male	$\bar{X}$	61.72^{b}	11.00	23.45^{a}
		Sd	1.50	0.26	0.86
	Female	$\bar{X}$	64.81^{a}	10.58	20.86^{b}
		Sd	2.07	0.85	1.49
E-II	Male	$\bar{X}$	62.19^{b}	11.08	23.21^{a}
		Sd	1.61	0.73	1.38
	Female	$\bar{X}$	62.35^{b}	10.95	22.91^{a}
		Sd	1.95	0.51	1.16

$\bar{X}$ - Average, Sd - Standard deviation
Different superscripts (a, b) indicate a statistically significant difference between groups (P <0.05)

Based on the analysis of the data from table 2, it can be concluded that the only change in the concentration of the protease enzyme (200 mg Ronozyme ProAct/kg feed and 4% less crude protein than the control group or 300mg/kg feed and 6% less crude protein than the control) had a significant effect (P<0.05) on the percentage of class I meat and class III meat, while there were no differences between the standard feeding treatment (C group) and the E-I group, i.e. E-II group. Female broilers from the E-I group had a higher percentage of class I meat and a lower percentage of class III meat compared to females from the E-II group. In similar research, Dosković et al. (2020) report a significant effect of protease (P<0.05) in the Master Gris hybrid on the percentage yield of wings - class II meat, while Mahendran et al. (2022) determined that protease supplementation to broiler fed with a corn-soybean

meal based normal and low crude protein diets did not have any effect on carcass characteristics.

The effect of sex was manifested in the percentage of class I meat and class III meat only in the E-I group (P<0.05), while there was no effect of sex on the percentage of class II meat (P>0.05). Namely, female chickens in the E-I group had a higher percentage of class I meat, and a lower percentage of class III meat compared to male broilers. Similar results about the percentage of class II meat are reported by Young et al. (2001) and Kamporn et al. (2022), while Bogosavljevic-Bošković et al. (2011) and Dosković et al. (2020) determined that the percent yield of class I and III meat was not affected by sex (P>0.05).

Conclusion

Based on the analysis of data on weight and percentage of individual class meat, it can be concluded that the proposed substitution of the standard diet for fattening broilers with a diet that contained 4% less crude protein than the control (C) and was supplemented with protease (Ronozyme Pro Act) at a concentration of 200mg/kg feed did not affect the examined carcass quality parameters (P>0.05), consequently, differences appeared only in the percent yield of class I and III meat between E-I (200mg Ronozyme Pro Act/kg feed) and E-II (300mg Ronozyme Pro Act/kg feed) feeding treatments (P<0.05).

The sex of the chicken affected the mass of all classes of meat and the percentage of class I meat and class III meat (P<0.05), while there were no differences only in the percentage of class II meat (P>0.05).

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RELATIONSHIPS BETWEEN SERUM ENZYME ACTIVITES IN THE MILK AND BLOOD IN DAIRY COWS DURING DIFFERENT STAGE OF LACTATION PERIOD

Radojica Đoković[1], Biljana Anđelić[2], Marko Cincović[3], Miloš Ži. Petrović[1], Aleksandar Čukić[4], Miroslav Lalović[5]

Abstract: This study aimed to determine blood and milk enzyme activites as indicators of liver function and their correlations in dairy cows during different stage of lactation period. Blood and milk samples were collected from 100 Holstein dairy cows during morning milking. The cows were allocated to four groups according to the production period, including cows in early (n = 18), full (n = 26), mid (n = 25) and late (n = 31) lactation. The value of serum enzyme activites of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and lactate dexydrogenase (LDH) in the blood and milk were determined. The activities of blood and milk serum AST, ALT, ALP and LDH were significantly higher ($P< 0.01$) in early and full lactation cows than in the other two groups of cows, suggesting mild fat infiltration of liver cells. The enzyme activities in milk were positively correlated with those in the blood ($P< 0.01$). In conclusion, similar changes in blood and milk serum enzyme activites during lactation and milk to blood correlations confirm that milk has great potential in predicting of blood metabolites and metabolic status of cows.

Keywords: cows; blood, milk, enzyme activites, lactation

Introduction

The metabolic profile, a series of specific blood analytical tests, is routinely used to reveal metabolic problems in dairy cattle (Oetzel, 2004; Stengarde et al., 2008; Gross et al., 2011). Evaluation of the blood and milk biochemical

[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia (radojicadjokovic@gmail.com)

[2]University of Nis, Faculty of Agriculture – Krusevac, Kosanciceva 7, 37000 Krusevac, Serbia

[3]University of Novi Sad, Faculty of Agriculture, Novi Sad, Square Dositeja Obradovica 7, 21000 Novi Sad, Serbia

[4]University of Pristina, Faculty of Agronomy in Lesak, Jelene Anzujske bb, 37200, Lesak, Serbia

[5]University of East Sarajevo, Faculty of Agroculture, Vuka Karadžića 30, East Sarajevo, Bosnia and Herzegovina.

parameters to assess the animal health and milk yield has always been interesed by authors and the various discrepancies have been observed in both blood and milk yield results (Nozad et al., 2011; Jozwik et al., 2012).

Milk parameters originate from blood and food component and clarifying the appropriate relationships among these parameters individually in food, blood and milk are useful in understanding the health and production status in animals (Jozwik et al., 2012; Liu et al., 2012, 2013; Ghadaa, 2014).

Major health disorders in high-yielding cows occur around parturition and during lactation. Metabolic conditions of negative energy balance (fasting, parturition and lactation) lead to an increased uncontrolled rate of mobilization of body fat and its increased accumulation in liver cells, resulting in disturbance of the physiological and morphology integrity of the liver (Jorritsma et al., 2001; Overton and Waldron, 2004; Bobe et al., 2004).

Fatty liver and diffuse infiltration of hepatocytes involve cell membrane damage and hepatocyte destruction accompanied by the release of cytoplasmic enzymes (ALT, AST, GGT, LDH), the activity there of in the blood being considerably elevated (Oezel, 2004; Stojevic et al., 2005; Lubojacka et al., 2005; Djokovic et al., 2018; 2019).

Blood plasma and serum ALT, AST, ALP and GGT activities were reported to be useful indicator of liver function for postpartum dairy cows (Bobe et al, 2004; Stojevic et al., 2005 Mordak et al.,2020). While little information is available concerning about the activity changes of ALT, AST, GGT and ALP in milk. The activities of these enzymes were monitored in milk and blood serum of cows and results of correlation analysis and regressive models showed a close relation between them (Liu et al., 2012, 2013; Ghadaa, 2014).

More practical attention has been given to detection of enzyme activity in milk and many enzymes have been proposed and listed a sreliable markers for early diagnosis of subclinical desease (Babaei et al., 2007; Katsoulos et al., 2010; Djokovic et al., 2019).

The objective of this study was to determine correlation between serum blood and milk enzyme activities as indicator of liver function in the different stage of lactation in the dairy cows.

Materials and methods

Animals and study design

A total of 100 dairy cows were randomly selected from the same Holstein herd containing 1200 cows (FARM: Kraisnik, Backa Palanka, Vojvodina, Serbia).

Clinically healthy cows were allocated to four experimental groups: Group 1 – early lactation cows (n = 18), from 1 to 49 days of lactation; Group 2 – full lactation cows (n=26), from 50 to 109 days of lactation; Group 3 – mid-lactation cows (n=25), between 110 and 209 days of lactation; and Group 4 – late lactation cows (n=31), from 210 to 305 and more days of lactation. The cows were high-yielding, aged 4 years on average (with an average of 2.7 lactations), with a preceding lactation of about 8500L (average weekly yield was 26.5 L/cow/day). The average body condition score (BCS) was 3.36±0.55 for all experimental cows. The experimental cows were housed in free-stall barns. Diet and housing conditions were adapted to the purposes of the experiment, with diet tailored to the cows' energy requirements during different periods of lactation using NRC standards (NRC, 2001).

Blood analysis

Blood samples were taken 4 to 6 h after milking and feeding from the coccygeal vein into evacuated serum separator tubes. After clotting for 3 h at 4 °C and centrifugation (1500 G, 10 min), blood sera were analyzed for the following biochemical parameters: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) and lactate dexydrogenase (LDH), which were determined by colorimetric kits (Biosystem, Spain and Randox, Carlisle, UK) and a Chemray spectrophotometer (Rayto, Shenzhen, China).

Milk analysis

Milk samples were collected during morning milking into tubes with and without additives on the same day blood was sampled. Upon serum separation, milk was subjected to biochemical tests for the determination of the enzymes (AST, ALT, ALP, GGT, LDH). Milk serum was separated after centrifugation at 10,000g for 30 minutes, and was transferred to new tubes for analysis. The biochemical reagents and apparatus used for milk serum analysis were the same as for blood serum. All analyses were performed at the Laboratory of Pathophysiology, Department of Veterinary Medicine, University of Novi Sad.

Statistical analysis

The statistical analysis of the obtained data was carried out by ANOVA-procedure (Statgraphic Centurion, Statpoint Technologies Inc.Warrenton, Va, Virginia, USA). The effect of lactation period on blood and milk serum enzyme activites was examined by an ANOVA analysis coupled with an LSD post hoc

test. Associations between milk and serum enzyme activites were determined by Pearson's coefficient of correlation. The SPSS statistics software (IBM, USA) was used.

Results and discussion

The peripartal and early lactation periods were considered as time periods that have the potential to enhance lactation performance. Modern dairy farming often results in forced milk production, giving rise to metabolic disorders in cows. In order to predict such disorders and related subclinical diseases, it is necessary to establish the physiological ranges of biochemical parameters in a clinically healthy herd (Reist et al., 2002; Radostits et al., 2000; Pires et al., 2022).

Lactation period showed a statistically significant influence on blood and milk biochemical parameters. In most cases in this experiment, blood and milk serum enzyme activites during early lactation were significantly different from those in the other periods of lactation (Tables 1 and 2).

Table 1. Blood metabolites in early (Group 1), mid (Group 2), full (Group 3) and late (Group 4) lactation dairy cows. Results are expressed as mean ± standard deviation (SD).

	Group 1	Group 2	Group 3	Group 4		
AST (IJ/L)	134.8±37.3	100.9±30.1	99±37	95.2±31.7	P<0.001	1:2,1:3,1:4
ALT (IJ/L)	59.16±19.87	36.00±9.46	28.60±5.63	28.64±6.85	P<0.001	1:2,1:3,1:4, 2:3,2:4
LDH (IJ/L)	1795±942	1647±329	1389±224	1312±285	P<0.001	1:3,1:4, 2:4
ALP (IJ/L)	108.17±27.9	85.88±16.7	75.84±15.8	71.00±18.3	P<0.001	1:2,1:3,1:4
GGT (IJ/L)	24.61±6.1	21.19±6.1	23.24±10.8	27.19 ±6.6	P<0.05	2:4

ST – aspartate aminotransferase; ALT – alanine aminotransferase; ALP – alkaline phosphatase; GGT – gamma-glutamyl transferase; LDH – lactate dexydrogenase.

Table 2. Milk metabolites in early (Group 1), mid (Group 2), full (Group 3) and late (Group 4) lactation dairy cows. Results are expressed as mean ± standard deviation (SD).

	Group 1	Group 2	Group 3	Group 4		
AST (IJ/L)	125.7±71.7	62.3±35.1	59.1±32.4	69.6±58.1	P<0.001	1:2,1:3,1:4
ALT (IJ/L)	54.8±24.9	36.3±11.2	28.3±6.9	28.4±7.03	P<0.001	1:2,1:3,1:4, 2:3,2:4
ALP (IJ/L)	947.1±543.3	629.5±338.5	568.2±325.8	670.8±358.7	P<0.05	1:2,1:3,1:4, 2:4,3:4
GGT (IJ/L)	561.9±217.1	446.9±161.7	571.3±325.8	670.8±135.9	P<0.01	2:3,2:4
LDH (IJ/L)	316.4±156.4	205.6±132.6	200.6±123.2	213.6±117.1	P<0.05	1:2,1:3,1:4

AST – aspartate aminotransferase; ALT – alanine aminotransferase; ALP – alkaline phosphatase; GGT – gamma-glutamyl transferase; LDH – lactate dexydrogenase.

Table 3 shows the coefficients of correlation between blood and milk biochemical parameters calculated for all cows in this experiment.

Table 3. Correlation between milk composition and diagnostic blood metabolites

	Milk AST	Milk ALT	Milk ALP	Milk GGT	Milk LDH
Blood AST	**0.450****	0.185	0.035	-0.115	0.029
Blood ALT	**0.266****	**0.649****	**0.262****	-0.004	0.161
Blood LDH	0.06	**0.347****	0.073	-0.135	0.116
Blood ALP	**0.399****	**0.492****	**0.343****	0.01	**0.338****
Blood GGT	0.036	0.107	0.163	**0.211***	-0.017

* – statistically significant correlation (P< 0.05); **– highly significant correlation (P< 0.01)

During the first month of lactation, 5–10% of high-yielding dairy cows suffer from severe hepatic lipidosis and 30–40% of cows develop mild hepatic lipidosis (Bobe et al., 2004), which indicates that almost 50% of these cows are at risk for metabolic disorders. Fatty infiltration of the liver causes lesions in the hepatic tissue and a general increase in the levels of the enzymes indicating

hepatocyte injury, i.e. AST, GGT, and GLDH (Pechova et al., 1997; Lubojacka et al., 2005; Stojevic et al., 2005; Le Blanc, 2010; Djokovic et al., 2019; Mordak et al., 2020).

In this experiment, the activities of blood and milk serum AST, ALT, ALP and LDH were significantly higher ($P< 0.01$) in early and full lactation cows than in the other two groups of cows, suggesting fat infiltration of liver cells and a release of these enzymes in circulation as induced by lipomobilization. Changes in blood and milk AST, ALT, ALP, LDH and GGT activities at different lactation stages indicated a mild degree of hepatic lesions in early lactation cows, probably due to fat infiltration.

This statement was confirmed by strong significant positive correlations between blood and milk serum AST ($r= 0.450$; $P< 0.01$), ALT ($r= 0.649$; $P< 0.01$), ALP ($r= 0.344$; $P< 0.01$) and GGT ($r= 0.211$; $P< 0.05$) activities in this study (Table 3).

These results are supported by the reports of other authors (Liu et al., 2012, 2013; Ghadaa, 2014; Djokovic et al., 2018; Benedet et al., 2019), who showed that milk enzyme activities can be good indicators of lipid mobilization and ketogenesis in cows during lactation for early detection of subclinical disease. The high correlation coefficient of the work agrees with the finding of Liu et al., (2012), and the high significance arises due to the large number of samples examined in this experiment. In the experiment in ewes, the relations between indicators of milk composition, milk production and blood indicators as well as their mutual connections indicate the justification of using the analysis of liver status indicators in ewes (Antunovic et al., 2022).

Conclusion

Based on changes in blood and milk serum enzyme activites metabolites at different stages of lactation, the results of this study indicated a mild degree of hepatic lesions in early lactation cows, probably due to fat infiltration. The results showed that changes in blood and milk serum enzyme activites over the entire course of lactation in dairy cows and their relationships can be used to monitor metabolic status in dairy cows at the herd level, and can serve as excellent milk biomarkers for the early detection of subclinical metabolic disease.

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ASSESSMENT OF GROUNDWATER QUALITY FOR IRRIGATION IN NORTHERN VOJVODINA

Milica Vranešević[1], Atila Bezdan[1], Boško Blagojević[1], Radovan Savić[1], Radoš Zemunac[1], Gordana Šekularac[2], Miroljub Aksić[3]

Abstract: Agriculture is one of the largest consumers of water and the importance of its quality need to be usable, because the consequences of applying water of unsuitable quality are permanent and far-reaching. Assessment of groundwater usability should be performed according to available parameters. For the needs of classifications, water parameters were analyzed all cations, anions, total dissolved salt and electrical conductivity. According to all the classifications, the analyzed groundwater can be a good source of water for irrigation in terms of its quality, but with control and appropriate measures.

Keywords: water quality, groundwater, irrigation, Vojvodina

Introduction

Under the influence of increasingly frequent extreme hydrometeorological phenomena such as droughts, more attention should be essentially taken into the full consideration to water resources, both from the aspect of quantity and from the aspect of quality. Agriculture is one of the largest consumers of water and the importance of its quality should be emphasized, because the consequences of applying water of unsuitable quality are permanent and far-reaching. It is known that the composition of irrigation water has a major impact on soil characteristics, on yield and quality of cultivated plants, and on irrigation equipment (Ayers and Westcot, 1976; Bortolini et al., 2018; Bauder et al., 2011; Fipps, 2003). Excess amount of some ions in irrigation water could causes salinity, sodicity and permeability problems in the root zone affects plant growth and crop yield. An increase in total salts, individual ions and their unfavorable ratio increases the risk of unwanted consequences. Striving for sustainable management and agricultural production,in order to avoid or at least reduce the negative effects of the use of potentially unsuitable water, it is

[1]University of Novi Sad, Faculty of Agriculture, Department of Water Management, Trg Dositeja Obradovića 8, Novi Sad, Serbia (milica.vranesevic@polj.edu.rs);
[2]University of Kragujevac, Faculty of Agronomy Čačak, CaraDušana 34, Čačak, Serbia;
[3]University of Priština, Kosovska Mitrovica, Faculty of Agriculture, Kopaonička nn, Lešak, Serbia.

precisely the analysis of these parameters from the aspect of agriculture that is necessary. The assessment of groundwater usability should be performed according to parameters that have a direct impact on the soil in terms of its productivity and in accordance with its characteristics. Irrigation equipment could be damaged by the usage of unsuitable irrigation water, which primarily refers to emitter congestion and this result in poor uniformity of watering, which leads to uneven plant development. Regarding the impact on the equipment of the irrigation system, suspended matter, bicarbonates, sulfides, manganese and iron are distinguished (Bortolini et al., 2018). In order to prevent the destructive impacts, water quality assessment should become a necessary measure for production under irrigation systems (Joshi et al., 2009). Numerous classifications have been developed to assess the quality of irrigation water under different conditions, however, the classifications known in Serbia, the Nejgebauer classification, and in the world such as the FAO classification (Ayers and Westcot, 1976) and the USSL classification (Richards, 1954) are commonly used. The aforementioned classifications were also applied in this paper and the assessment of groundwater quality was performed from the aspect of use for irrigation.

The aims of present study, which has been carried out during ten years monitoring period, were to determine characteristic of groundwater in a subjected area and its usability assessment for irrigation purposes using the most common classifications in Serbia and also common in the word.

Materials and methods

The quality of groundwater on the measuring points from which the analyzed samples were taken were Subotica-Mikićevo, Aleksa Šantić and Njegoševo. Those measuring points were obtained in the period from 2011. to 2020. and assessment of groundwater quality were done. Data on physical and chemical parameters of sample were taken from the Hydrological Yearbook of water quality from the Agency for the protection of the environment (SEPA, 2011-2020).

Statistical analysis of physical and chemical parameters of sampled water was performed on the basic parameters of water quality (total dissolved salts, electrical conductivity, as well as cations and anions). The analyzed quality parameters and their values of minimum, maximum, means and standard deviations (SD) are given in Table 1. A detailed analysis of the parameters required for the application of the three water classification classes for irrigation

has been carried out. First used classification is categorization of water according to Nejgebauer which is adapted to the natural conditions of Vojvodina (Belić et al., 2011), second is FAO classification (Ayers and Westcot, 1976) and US Salinity Laboratory classification (USSL) (Richards, 1954). The basis of these classifications is the analysis of potential problems of salinization and alkalization, i.e. analysis of the concentration of total salt in water and of sodium, or its relation to divalent cations ($Ca^{2+}$and Mg^{2+}), and FAO classification provides more detailed analyses.

Table 1. Analyzed parameters on the measuring points in period 2011-2020

	Subotica-Mikićevo				**Aleksa Šantić**				**Njegoševo**			
Parameter	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**
Ca^{2+} (mg/l)	157.2	87	121.97	27.06	107	90.9	95.55	4.73	121.2	75	92.52	12.93
Mg^{2+} (mg/l)	84	41.8	59.46	15.20	33	18	26.17	3.70	49	8.8	30.6	10.32
Na^{+} (mg/l)	72.5	54	63.4	6.18	59.5	41.5	49.39	5.98	193.5	48.8	71.09	43.52
K^{+} (mg/l)	2.6	1	1.78	0.51	2	1	1.64	0.35	1.8	0.9	1.49	0.27
HCO_3^{-} (mg/l)	608	511	556.6	40.44	508	479	490.9	9.87	705	391	437.7	95.04
Cl^{-} (mg/l)	162	33	91.19	52.21	31.9	18	23.11	4.87	71.8	48.3	60	8.16
SO_4^{2-} (mg/l)	150	53	95.4	30.75	33	10	23.2	8.01	72	38	52.1	12.06
NO_3-N (mg/l)	0.5	0.03	0.128	0.14	0.31	0.02	0.097	0.09	23.65	0.08	3.036	7.29
EC (□S/cm)	1586	880	1188.2	275.63	828	708	776.5	31.25	1407	787	895.4	185.22
TDS (mg/l)	990	588	759.4	160.51	525	451	484.2	24.80	895	484	560.9	118.61
pH value	7.5	7.1	7.34	0.11	7.5	7.1	7.272	0.13	7.67	7.04	7.409	0.20

Results and discussion

The obtained results indicate that, according to the Nejgebauer classification, on the measuring point Subotica-Mikićevo, there was fluctuation in the water quality from first to third class of water for irrigation.

Graph 1. Percentile representation of individual water classes on the measuring points according to Nejgebauer classification, 2011-2020

On measuring point Aleksa Šantić water quality were uniform through entire analyzed period, while on the measuring point Njegoševo, the second and fourth class of irrigation water appears. The percentage representation of certain classes during the analyzed period according to Nejgebauer's classification is shown in the Graph 1.

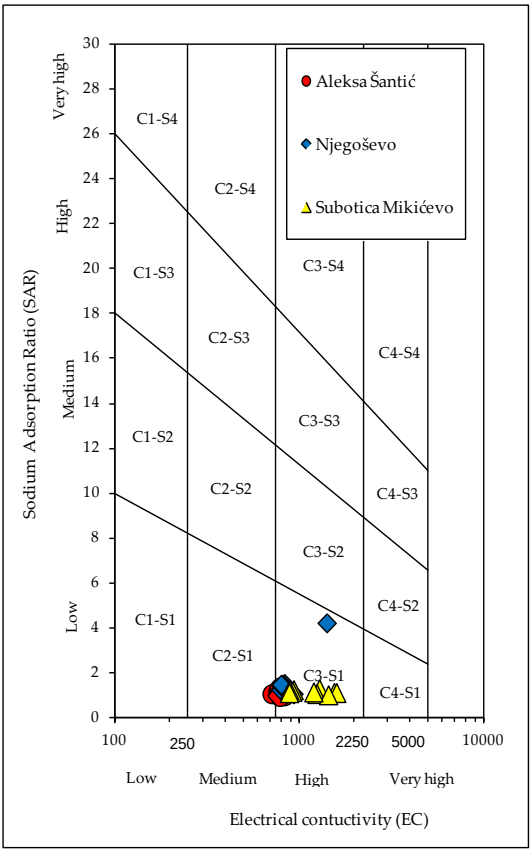
According to the FAO classification criteria results during the observed period are presented in Table 2. On all measuring points water quality in terms of salinization not changed from first class, which has no limitations. According to the influence of water on infiltration, the quality condition is similar in sense of changing on all measuring points, but it is it is characterized by belonging to class II, moderate use restriction. Special attention should be paid to the possible effects concentrations of Na^+ on measuring point Subotica-Mikićevo and Njegoševo.

Table 2. Percentile representation of individual water classes on the measuring points according to the FAO classification, period 2011-2020

	Subotica-Mikićevo		**Aleksa Šantić**		**Njegoševo**	
Potential problems	Class (%)		Class (%)		Class (%)	
Salinity	**II**	100	**II**	100	**II**	100
Infiltration	**I**	100	**I**	100	**I**	100
Toxicity of Na^+	**I**	70	**I**	100	**I**	90
	II	30			**II**	10
Toxicity of Cl^-	**I**	60	**I**	100	**I**	100
	II	40				

Measuring point Subotica-Mikićevo concentration was within the first class of no limitations in using water for irrigation in 70% of analyzed samples, with occasional occurrences of the need for moderate use restriction (II class) in 30% of analyzed samples. Similar occasions were on measuring point Njegoševo but in other percentage. Concentration was within the first class of no limitations in using water for irrigation in 90% of analyzed samples, with occasional occurrences of the need for moderate use restriction (II class) in 10% of analyzed samples. On measuring point Aleksa Šantić concentrations of Na^+ are within the limits in which they do not exhibit toxic effects on plants. Another specificity of FAO classification is the influence of chloride ions. On measuring point Subotica-Mikićevo concentration was within the first class of no limitations in using water for irrigation in 60% of analyzed samples, with

occasional occurrences of the need for moderate use restriction (II class) in 40% of analyzed samples. On measuring point Aleksa Šantić and Njegoševo concentrations of Cl^- are within the limits in which they do not exhibit toxic effects on plants.



Graph 2. Water classes according to USSL classification all measuring points, 2011-2020

USSL classification, based on the value of electrical conductivity and the Sodium Adsorption Ratio, water samples on measuring point Aleksa Šantić were classified mainly in the C3-S1 class, more precisely about 90% of the samples, and about 10% in the C2-S1 class. On other two measuring point all samples were classified in C3-S1 class. Graph 2 shows that the EC values varied from lower to upper limit of the class, while all SAR values are closer to the lower limit value of the class. USSL classification classifies water the most of

samples in class C3-S1, i.e. "Salty" water without a significant risk of the effect of adsorbing harmful sodium in terms of alkalization. In terms of total salt therefore measures such as choice of cultures resistant to salt, and the inability to use this water on naturally poorly drained soils are necessary. Due to the appearance of class C3 in the irrigation season, regular controls and measures must be in line with assessment of groundwater quality.

Conclusion

Assessment of groundwater qualityaccording to all water classifications, the analyzed groundwater samples are usable for irrigation. The results according to the Nejgebauer classification adapted to the conditions of Vojvodina are most favorable for use. According to this classification, the quality of Subotica-Mikićevo and Aleksa Šantić is in I and II class about 80% to 100% of the samples, the remaining samples were "unsuitable" quality (III and IVclass). All three measuring points require control over the content of total salt, due to the impact on soil and plants, as well as the ratio of sodium concentration to calcium and magnesium (SAR value), due to a moderate usage of water to the salinity properties of the soil. Special control is needed on the concentration of Na^+ and Cl^-, from which there is a risk of occurrence of various adverse effects on the soil, irrigated plants and irrigation equipment. USSL classification classified water mainly to C3-S1 class which means that the water can be used for irrigation with mandatory control during the irrigation season. These results of research can contribute to a better understanding of the selection of sources for irrigation in accordance with its quality and indicate the usability of irrigation water.

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CULTIVATION, IMPORTANCE AND POSSIBILITES OF APPLICATION OF MEDICINAL PLANTS IN MEDICINE

Ljubica Šarčević-Todosijević[1], Kristina Vojvodić[1], Bojana Petrović[1], Vera Popović[2], Vladimir Filipović[3], Ljubiša Živanović[4], Jelena Golijan[4], Marko Burić[5]

Abstract: Medicinal and aromatic plants are intensively studied and applied in traditional and official phytotherapy. Medicinal plants can be cultivated or collected from nature. The pharmacological activity of plants is mainly due to the products of secondary metabolism. Recently, more and more attention has been paid to the study of the chemical structure and pharmacological activity of herbal drugs, which contain polyphenols due to their antioxidant and anti-inflammatory effect, and thus a beneficial effect on health.

Keywords: medicinal plants, importance, cultivation, biological activity, application in medicine.

Introduction

In the production of food on the planet level, the primary role belongs mainly to angiosperms, but this group of plants also includes species that are intensively used in pharmacy and medicine (Jančić, 2004; Šarčević-Todosijević et al., 2018). The cultivation of medicinal plants is no different from the cultivation of other crops, and in order to achieve optimal yields, optimal habitat conditions are necessary, as well as the timely application of agrotechnical measures. Medicinal plants can be cultivated or collected from nature (Kovačević, 2004). The area of the Balkans is very rich in medicinal plant species, but due to favorable climatic conditions, they can also be successfully grown on plantations (Šarčević-Todosijević et al., 2018; Šarčević-Todosijević et al., 2022a).

[1]High Medical and Sanitary College of Vocational Studies "Visan", Tošin bunar, 7a, Belgrade, Serbia (ljsarcevic@gmail.com)
[2]Field and Vegetable Crops Institute, Maksima Gorkog 30, 21000 Novi Sad, Serbia
[3]Institute for Medicinal Plant Research "Dr Josif Pančić", Belgrade, Serbia
[4]University of Belgrade, Faculty of Agriculture, Nemanjina 6, Zemun, Belgrade, Serbia
[5]University of Belgrade, Faculty of Medicine, Belgrade, Serbia and Health Center, Danilovgrad, Montenegro

Medicinal and aromatic plants are intensively studied and applied in traditional and official phytotherapy, but also in food production as spices and preservatives. The biological effect exerted by medicinal or aromatic plant species mainly depends on the main constituents of each species, but also on the amount of these constituents in the plant. The products of secondary metabolism are mainly responsible for the pharmacological activity of plants. Recently, more and more attention has been paid to the study of the chemical structure and pharmacological activity of herbal drugs containing polyphenols because their antioxidant and anti-inflammatory effects, and thus their beneficial effects on health. Epidemiological studies indicate that the long-term use of plant polyphenols in the diet has a preventive effect on the occurrence of cancer, diabetes, inflammatory, cardiovascular, neurodegenerative diseases, as well as aging-associated diseases. In addition to antibacterial and antifungal properties, natural components of plants are an important source for developing new antiviral drugs due to their availability and low side effects (Beckman, 2000; Kovačević, 2004; Arts and Hollman, 2005; Đorđević et al., 2020; Šarčević-Todosijević et al., 2022b; Popović et al., 2018a; 2018b; 2019; 2020; 2021).

Material and Methods

In this study an analysis of the production of medicinal plants was also done. On the desk research is based. A number of scientific papers results of domestics and foreign authors in the thematic fields is also analyzed and quoted. The analytical-synthetic method was used during the discussion and formulation of conclusions.

Results and discussion

In global food production, the primary role belongs to plant production, with the most important being angiosperms. Angiosperms also include species that are intensively used in pharmacy and medicine. Whether it is crop production for food or medical purposes, optimal crop yields can only be obtained through agricultural production based on scientific principles (Jančić, 2004; Đukić et al., 2007; Šarčević-Todosijević et al., 2018; Šarčević-Todosijević and Vojvodić, 2020). Cultivation of medicinal plants is not significantly different from cultivation of any other crop. In order to achieve optimal yields of medicinal plants, as well as the synthesis of pharmacologically active ingredients, optimal conditions of the habitat, especially the soil, are necessary.

According to the data of the Statistical Office of the Republic of Serbia, the areas under medicinal and aromatic plants in the period from 2002 to 2006 tend to decrease, from 1.832 ha in 2002 to 1.211 ha in 2006. From 2007 to 2009, the areas under medicinal and aromatic plants increased, and in 2012 there was a decrease and these areas amounted to only 1.337 ha (table 1).

Table 1. Production of medicinal and aromatic herbs in Serbia

Year	2002	2006	2012	2022	Interval variation
Area, ha	1832	1211	1337	1300	621

In cultivation of medicinal plants, care should be taken in the selection of the most suitable taxon (species or variety) for cultivation in a certain region, selection of soil, cultivation and preparation of the soil, selection of the optimal way to establish plantations (type of planting material and planting time), application of agrotechnical measures for cultivation and protection of the plants. Plants should be harvested at the stage of ontogenetic development, in which the quality of the drug is the most optimal. Some medicinal plants cannot be cultivated as plantations, because they produce pharmacologically active substances in the required quantities only in natural habitats. Such plants must be collected from nature (Kovačević, 2004). The Balkans is very rich in medicinal plant species. In the Košutnjak locality, Šarčević-Todosijević et al. (2018) collected and determined 32 plant species within 24 angiosperm families. All collected species show wide range of pharmacological activities and are used in phytotherapy (Šarčević-Todosijević et al., 2018; Šarčević-Todosijević et al., 2022a).

However, in the climatic conditions of the Balkans, medicinal herbs can be cultivated successfully. Miloradović (2018) investigated the impact of agro-ecological conditions in southern Banat and Pomoravlje on morphological characteristics, herb yield and quality of essential oil obtained by distillation of fresh immortelle biomass (*Helichrysum italicum*). These studies have shown that by choosing the most favorable system of production technology, commercial yields can be expected already from the second year of cultivation, as well as that Italian immortelle (*Helichrysum italicum*) can be successfully grown in continental areas, in this case in the hilly areas of Central Serbia, as well as in the plains of Vojvodina (Miloradović et al., 2018).

Filipović et al. (2021) conducted the first published research in the Republic of Serbia about the vegetative propagation of Pannonian thyme (*Thymus pannonicus*). In addition to morphological polymorphism, species of the genus

Thymus are characterized by a large chemical polymorphism in the composition of the essential oil, which exhibits strong inhibitory activity against *Helicobacter pylori* and other bacteria, as well as against *Candida albicans*. It is used in the auxiliary therapy and prevention of gastritis and in alleviating diseases of the respiratory tract and the gastrointestinal system. In the paper, they examined the impact of the application of three different phytohormones (INCIT2, INCIT 5 and INCIT 8, all based on α-Naphthalene Acetic Acid (NAA), with the cuttings of two forms of Pannonian thyme (the L-16 form, with hairy leaves, and the L-9 form, with hairless leaves), established during two periods (March and May), on the percentage of the rooted cuttings and the morphological properties of the seedlings. The variant without the application of the phytohormones was taken as the control. Based on the achieved results, Filipović et al. (2021) point out that INCIT 2 proved to be the most suitable phytohormone for the rooting of Pannonic thyme cuttings, with the average percentage of rooted cuttings of 61.3%. Thelowest rooting percentage was recorded in the control variant, only 29.4%. Satisfactory rootingwas found in the cuttings treated with INCIT 8 (57.6%). The L-16 form cuttings showed a higher rooting rate, an average of 53.8%, whereas the L-9 form cuttings had a lower average percentage of rooted cuttings (45.0%) for both plant establishment periods. Greater success and quality in the rooting of cuttings was recorded in the second (May) period of plant establishment, averaging 58.1%, which was higher by 17.4% than the percentage of rooted cuttings in the first establishment period (40.7%) (Filipović et al., 2021).

Popović et al. (2018) conducted an experiment with *Phacelia tanacetifolia* cultivar NS Priora in two variants: control, without nutrition and variant with nutrition, in organic cropping system. *Phacelia tanacetifolia* is commercial species, which has long been used for bee nutrition, and after acacia, it produces the most nectar. Foliar nutrition had a positive effect on all the tested characteristics. Analysis of variance was found highly significant effect of nutrition on leaf length, yield of biomass and plant height. This research indicated the justification of growing honey plants, especially the species *Phacelia tanacetifolia*, in a health-safe organic cultivation system (Popović et al., 2018a; 2020).

The pharmacological effect of medicinal or aromatic plant species mainly depends on the main constituents of each species, but also on the amount of these constituents in the plant. The therapeutic effects of medicinal plant species are very broad, they include antibacterial, antiviral, anti-inflammatory, antioxidant, anticancer, cytotoxic, spasmolytic, diuretic, sedative and many

other effects (Kovačević, 2004; Šarčević-Todosijević et al., 2019a,b; 2022b; Dročić et al., 2020; Đorđević et al., 2020; Popović et al., 2022; Petrović et al., 2022).

Polyphenols are a very important group of compounds, secondary metabolites of plants, due to their beneficial effect on health. Epidemiological studies indicate that the long-term use of plant polyphenols in the diet has a preventive effect on the occurrence of various diseases. As natural antioxidants, they are important in the prevention and treatment of cancer, inflammatory, cardiovascular and neurodegenerative diseases, and intake of fruits and vegetables, especially seeds and nuts, is associated with a lower risk of developing chronic and degenerative aging-associated diseases. Polyphenols include phenolic acids, flavonoids, coumarins, stilbenes and lignans, as well as polymerized forms such as tannins and lignin (Beckman, 2000; Kovačević, 2004; Arts and Hollman, 2005; Đorđević et al., 2020; Popović et al., 2020; 2021; Šarčević-Todosijević et al., 2022b).

The most common ingredients of *Ginkgo biloba* extract, one of the plant species most often used in phytotherapy, are also polyphenols (flavonoids). This plant has become increasingly important in the last thirty years, after the discovery and definition of a standardized extract EGb 761 (EGb 761 - standardized *Ginkgo biloba* leaf extract). This extract contains 24% heterosides and 6% ginkgolide and bilobalide. *Ginkgo* extract contains a number of active substances, which show effectiveness in the treatment of arterial and cerebrovascular insufficiency, dementia, vertigo, asthma, allergies, memory improvement, and acts as a strong antioxidant (Dubey et al., 2004; Kovačević, 2004).

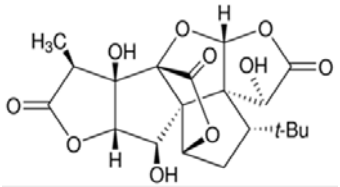

Figure 1. Ginkgolide A (Kovačević, 2004)

Defeudis (2002) from the *Institute for BioScience* in the USA, conducted studies on the effectiveness of the application of bilobalide. *In vivo* studies have indicated that systemically administered bilobalide can reduce cerebral edema, decrease cortical infarct volume in certain stroke models, and reduce cerebral ischemia. *In vitro* studies indicate that bilobalide has multiple mechanisms of action that may be associated with neuroprotection. These mechanisms include preservation of mitochondrial ATP synthesis, inhibition of apoptotic damage

induced by staurosporine or by serum-free medium, suppression of hypoxia-induced membrane deterioration in the brain and actions of increasing the expression of the mitochondrial DNA-encoded COX III subunit of cytochrome *c* oxidase and the ND1 subunit of NADH dehydrogenase Considering that it has multiple mechanisms of action, Defeudis (2002) points out that bilobalide could be useful in developing therapy for disorders involving cerebral ischemia and neurodegeneration.

Hop (*Humulus lupulus* L.) is also a source of numerous biologically active polyphenolic compounds. A significant compound, 8-prenylnaringenin (8-PN), belonging to the group of prenylated flavonoids, was shown to be a potent phytoestrogen. Due to its estrogenic effects, administration of 8-PN represents a novel therapeutic approach to the treatment of menopausal and post-menopausal symptoms. Application of 8-PN in the treatment of menopause has been clinically examined with good results. Other biological activities include the potential to prevent bone-resorption or tumor suppression. However, the use of phytoestrogens is often considered due to possible adverse effects associated with long-term use (Štulíková et al., 2018).

Pharmacologically active ingredients of plants that are widely used are also essential oils. Stevanović (2021) examined the antimicrobial and antiviral activity of essential oils of aromatic plant species *Origanum vulgare*, *Calluna vulgaris*, *Helichrysum italicum*, *Satureja montana* and *Thymus vulgaris* on strains of bacteria *Staphylococcus aureus* and fungi *Candida albicans* and *Candida krusei*. The antimicrobial activity of essential oils was tested by determining the minimum inhibitory concentration (MIC), as well as the potential of essential oils to reduce the biofilm of the tested strains of microorganisms. The essential oils showed significant antimicrobial activity, in the range of 0.078-5 mg/ml for *S. aureus* and 0.156-5 mg/ml for *C. albicans* and *C. krusei*. *Calluna vulgaris* essential oil (MIC=0.078 mg/ml) showed the highest antimicrobial activity against *S. aureus*, and *Helichrysum italicum* essential oil (MIC=0.156 mg/ml) against *Candida* sp. The significant potential of all oils in the reduction of biofilms produced by the tested types of microorganisms was also determined (Stevanović, 2021).

Due to the presence of various bioactive compounds, primarily essential oils, the plant species *Matricaria chamomilla* (chamomile) has anti-inflammatory properties (Kovačević, 2004). The phytochemistry composition of essential oils and extracts of *M. chamomilla* has been intensively studied and it has been determined that the plant contains over 120 constituents. Due to the mentioned chemical composition, *M. chamomilla* exhibits a wide range of biological activities, such as antioxidant, antibacterial, antifungal, antiparasitic,

insecticidal, antidiabetic, anticancer and anti-inflammatory (Mihyaoui et al., 2022). In addition to chamomile, numerous metabolites that exhibit strong antimicrobial, antiviral, anti-inflammatory, antioxidant, antimutagenic and anticancer biological activity have been isolated from the immortelle drug (*Helichrysum* sp.) (Miloradović, 2018).

Natural plant components are also an important source for developing new antiviral drugs due to their availability and low side effects. Most of the research is conducted on finding an antiviral drug that will inhibit various enzymes associated with the life cycle of the virus, so the connection between the structure of flavonoids and these enzymes was observed. Baicalin, isolated from the plant species *Scutellaria baicalensis*, inhibits HIV infection and viral replication. Different combinations of flavones and flavonols show synergism, so kaempferol and luteolin show a synergistic effect against Herpes Simplex Virus (HSV). Synergism can also occur between flavonoids and other antiviral agents (Hegi, 1981; Kovačević, 2004).

Conclusion

The area of the Balkans is very rich in medicinal plant species, but due to favorable climatic conditions, medicinal plants can also be successfully grown on plantations. The cultivation of medicinal plants is no different from the cultivation of other crops, and in order to achieve optimal yields, optimal habitat conditions are necessary, as well as the timely application of agrotechnical measures. The pharmacological effect that medicinal or aromatic plant species exhibit mainly depends on the main ingredients of each species, but also on the amount of these ingredients in the plant. Recently, more and more attention has been paid to the study of the chemical structure and pharmacological activity of herbal medicines, which contain polyphenols because of their beneficial effect on health. Natural components of plants are an important source for the development of new antiviral drugs.

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APPLICATION OF SEQUENTIAL EXTRACTION TO DETERMINE THE COMPOSITION OF ZEOLITE FOR ITS SAFE USE IN AGRICULTURE

Goran Petrović[1], Violeta Mitić[1], Jelena Nikolić[1], Milan Mitić[1], Marija Dimitrijević[2], Aleksandra Đorđević[1], Vesna Stankov Jovanović[1]

Abstract: Zeolites are alumo-silicate minerals, commonly used industry, medicine and agriculture. Their application in agriculture is based on the fact that they can increase crop yield and promote nutrient use efficiency. In order to determine is zeolite (clinoptilolite) safe for use in agriculture, regarding its elemental analysis, sequential extraction followed by Inductively coupled plasma - optical emission spectrometry analysis is performed. Sequential extraction perrmited insight into elemental composition of clinoptilolite, through various extraction phases – exchagable, carbonate bounded, metals bound to Fe and Mn oxides, metals bound to organic matter and residual phase. The most abundant elements in first phase are Ca, K, Mg and Na (with a concentration greater than 100 ppm). Similar observation wa made for second phase of sequential extraction, but in this phase high content of Mn (232.6±0.7 ppm) was also noticed. In those two phases, that represents cations available to plants, no toxic elements exceeded permissible values prescribed by Serbian law. However, he content of Be (1.822±0.002 ppm) in sample obtained from third phase exceeds the concentration limit prescribed by Serbian law, which might be potentially dangerous, since Be could be adopted by plant and transferred to human through food chain. Concentration of toxic Cd was twice as higher compared to law limitations in residual phase. Bearing in mind obtained results, clinoptilolite analyzed in this study could be used in agriculture, but with continuous soil properties monitoring, since toxic elements could be mobile under various reducing and pH conditions.

Keywords: zeolite, agriculture, sequential extraction, ICP OES

[1]University of Niš, Faculty of Sciences and Mathematics, Višegradska 33, 18 000 Niš, Serbia (peca@pmf.ni.ac.rs)

[2]University of Niš, Faculty of Medicine, Bulevar dr Zorana Djindjica 81, 18 000 Niš, Serbia

Introduction

Zeolite belongs to the family of natural silicate minerals, created by mixing volcanic lava with alkaline groundwater. For that reason, they are rich in aluminum, sodium and calcium and are known for their great absorption power. Zeolites have a porous structure that can absorb sodium, potassium, calcium, magnesium, but also heavy metals elements, such as mercury, lead, uranium. Their crystal grid consist of channels, which contain water and metal cations, that are easily exchangeable and are not part of the lattice. These channels are large enough to allow the passage of various chemical species (Maesen and Marcus, 2001).

Because of this characteristic, zeolite has found application in numerous branches of industry, medicine and agriculture. In agriculture, they are widely used as meliorants to improve soil quality, fertility, water and air permeability, ability to absorb and retain water in the soil. They are a source of slow release of potash and nitrogen, according to plant needs. They help development of the root system and are widely used in farming, vegetable growing, fruit growing, and viticulture as substrate for stimulating growth, increasing crop yields, and reducing costs (Sha et al., 2022).

The aim of this work is to determine the total content of elements in zeolite (clinoptilolite) is distributed between various phases. Knowing zeolite elements content is of great importance for its usage in agriculture, since it could contain toxic elements, which could be adopted by plant tissues, and further transported to human, through food chain. The fractionation of the total content of elements is carried out by sequential extraction (Tasić, 2016). This method provides a useful information about composition of a solid sample, such as the origin, form of finding elements, their mobility, biological and physical-chemical availability. Zeolite samples from two locations were subjected five stage sequential extraction and the obtained extracts were be analyzed for the presence and concentration of the following elements: Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, Pb, Si, V and Zn.

Materials and methods

The zeolite samples were collected in the vicinity of Vranjska Banja, in March 2022, near vilage Katalenac. Samples were homogenized and one sample was obtained. A small amount of sample (200 g) was crushed and sieved and then subjected to drying at 120 °C for 3 hours. Dry samples were stored in a desiccator until analysis.

Zeolite samples were analyzed by five stage sequential extraction, which was conducted in following steps:

Exchangeable metals (I): The sample (1 g) was weighed and mixed with 8 mL CH_3COONa (pH=8.2). Obtained mixture was mixed for 1 h at room temperature. Centrifugation was performed at 3800 rpm for 10 minutes, after of which the extract was transferred to a 25 mL vessel. The precipitate was washed with deionized water and tranfered to the next phase.

Metals bounded to carbonates (II): 10 mL of acetate buffer (pH=5) was added to the solid residue from the first stage The samples were mixed for 5 h at room temperature temperature. Extract was separated from the solid and transferred to a 25 mL vessel. The precipitate was washed with deionized water and tranfered to the next phase.

Metals bound to Fe and Mn oxides (III): 2 OH·HCl solution was added to the residue from the previous phase. The extraction is carried out at a temperature of 96±3 °C with occasional stirring during the 6 hours, with a reflux condenser. The extract was separated from the solid phase by centrifugation and transferred to a 25 mL vessel.

Metals bound to organic matter (IV): 0.02 mol dm^{-3} HNO_3 and 30 % H_2O_2 (pH=2) were mixed with the solid residue from the previous stage. Sample was heated to 86±2 °C for 2 h, with reflux condenser. After that, another portion of of 30 % H_2O_2 was added, and the samples were heated for another 3 h at the same temperature. After cooling, 5 mL of 3.2 mol dm^{-3} CH_3COONH_4 was added to the sample. The samples were mixed for 30 minutes and extract was separated from the solid phase by centrifugation and transferred to a 25 mL vessel.

Residual phase (V): The solid residue from the previous step was subjected to extraction using a mixture of HF-$HClO_4$ and evaporated to dryness. The dry residue was dissolved in 12 mol dm^{-3} HCl and transferred to a 25 mL vessel and made up to the mark with deionized water.

Elemental analysis was performed on ICP-OES spectrometer iCAP 6000 Series (Thermo Scientific, Cambridge, UK).

Results and discussion

Sequential extraction provides insight into the bioavailability and mobility of elements in the samples. The concentrations of all analyzed elements for all stages of five-stage sequential extraction are given in Table 1.

Table 1. Elemental composition of analyzed zeolite (ppm)

Element	I	II	III	IV	V
Al	5.3±0.2	30.0±0.4	264.0±0.5	855±3	36865±138
As	0.20±0.06	0.26±0.01	4.06±0.09	0.97±0.09	21.8±0.2
Ba	9.06±0.08	15.5±0.1	49.4±0.4	40.9±0.2	505±6
Be	0.161±0.005	0.248±0.002	1.822±0.002	0.283±0.005	1.19±0.02
Ca	1857±21	2690±48	1661±12	962±33	2453±11
Cd	0.010±0.002	0.078±0.005	0.286±0.002	0.127±0.002	1.83±0.03
Co	0.017±0.007	0.035±0.007	1.26±0.01	1.985±0.005	2.86±0.04
Cr	0.035±0.001	0.474±0.005	0.80±0.01	0.46±0.01	6.5±0.1
Cu	0.414±0.005	0.73±0.03	5.14±0.04	3.35±0.02	4.09±0.03
Fe	1.81±0.01	20.71±0.08	1245±2	776±6	7787±127
Hg	0.19±0.01	0.22±0.07	0.273±0.004	0.27±0.04	0.027±0.007
K	1470±71	1279±9	387±36	376±4	8499±85
Mg	189.7±0.2	93±1	234±1	217±4	1445±14
Mn	3.70±0.03	232.6±0.7	715±7	200±2	61±1
Na	12472±783	11300±442	884±37	54.7±0.7	1577±27
Ni	0.058±0.005	0.088±0.005	1.53±0.01	1.252±0.002	1.94±0.05
Pb	0.15±0.02	0.69±0.03	7.98±0.01	6.18±0.08	25.6±0.5
Si	44±4	76.5±0.8	1322±4	1119±8	48±5
V	0.560±0.007	0.291±0.002	3.09±0.01	1.84±0.02	15±2
Zn	1.10±0.03	2.18±0.03	16.87±0.03	9.7±0.2	31.7±0.4

The elements from the first stage of extraction represent the most mobile and plant-available phase. Among the most abundant elements in this phase are Ca, K, Mg and Na (with a concentration greater than 100 ppm). Element with the highest concentration in this phase of sequential extraction is Na (12472±783). Sequential extraction, which provides metal concentrations in the appropriate fractions of the sample, is of great importance for examining the behavior of toxic, especially first stage of extraction, since this phase are exchangeable elements. It provides a more complete insight into the behavior of metals in the environment, for a longer period of time and under different environmental conditions, as well as artificially induced conditions. The content of toxic elements in this phase is generally low (below 0.5 ppm) and is within the permitted concentration values prescribed by the Regulation on the program of systematic monitoring of soil quality, indicators for assessing the risk of soil degradation and the methodology for developing remediation programs (Ministry of Environmental Protection, 2010). Metals bound to carbonates also represent elements that are available to plants. The most abundant elements in this phase are Ca (2690±48 ppm), K (1279±9 ppm) and Na

(11300±442 ppm). A high Mn content was recorded for this phase from extraction (232.6±0.7 ppm). As far as toxic metals are concerned, at this stage there is no exceed of the concentration limit values prescribed by the Regulation (Ministry of Environmental Protection, 2010). In third phase elements bound to iron and manganese oxides and hydroxides are extracted, and they can be mobile under reducing conditions. Si (1322±4 ppm) and Fe (1245±2 ppm) are the most abundant elements in this phase. The content of Be (1.822±0.002 ppm) exceeds the concentration limit prescribed by the Regulation (Ministry of Environmental Protection, 2010) which is 1.1 ppm. Only a few studies have documented the toxic effects of Be on plants, however plant products (fruits, grains...) may be the main source of Be toxicity in the human food chain (Tanveer and Wang, 2019). Metals extracted in fourth phase are bound to organic matter, as well as sulfides, and their release is influenced by soil redox potential and pH. The main elements present in this phase are Ca (962±33 ppm), Fe (776±6 ppm), Si (1119±8 ppm) and Al (855±3 ppm). Toxic metals in this phase are within the permissible concentration limits determined by the Regulation (Ministry of Environmental Protection, 2010). The residual phase represents metals that are structurally bound in silicates and is generally unavailable to plants. The most abundant element in the sample, in this phase is Al (36865±138 ppm). The concentration of toxic metals is the highest in this phase, compared to the other phases of the described extraction technique, with the exception of Hg. Only Cd exceeds the concentration limit prescribed by the Regulation (Ministry of Environmental Protection, 2010). Limit value for Cd prescribed by the Regulation is 0.8 ppm. Cd content in analyzed zeolite sample is 1.83±0.03 ppm, which is twice higher than permissible value. Higher values for Cd could be explained by traffic and combustion emissions. Other anthropogenic factors of environmental pollution with cadmium include mining and metal industry, landfills and sewage sludge as well as the use of mineral phosphate fertilizers (Fasih Ullah Haider et al., 2021). The presence of Cd through rock diagenesis is also not excluded, given that the "Grot" lead and zinc mine in Kriva Feja is located nearby. The mobility of Cd^{2+} and its availability to plants largely depend on the pH value and redox potential, as well as the microbiological activity in the soil. In plant cultures, Cd^{2+} toxicity reduces nutrient and water uptake and translocation, increases oxidative damage, disrupts plant metabolism, and disrupts plant morphology and physiology (Haider et al., 2021).

Conclusion

Zeolites are widely used in agriculture, as meliorants to improve soil quality, fertility, water and air permeability, ability to absorb and retain water in the soil. Using sequential extraction and optical emission spectrometry with inductively coupled plasma, the content of elements in natural zeolite was determined. Among the present elements, there are micro- and macronutrients, which are extremely important for the nutrition of plant species. Toxic elements can also be found in zeolite, which negatively affect the growth and development of the plant. Be was found in the third phase of sequential extraction, whereas Cd was found in the fifth stage, with values higher compared to ones prescribed by Serbina law regulations.

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INCLUSION COMPLEXES OF PESTICIDES IN HYDROXYPROPYL-β-CYCLODEXTRINE. EFFECTS ON THEIR WATER SOLUBILITY

Goran Petrović[1], Aleksandra Đorđević[1], Jelena Stamenković[2], Violeta Mitić[1], Jelena Nikolić[1], Milan Mitić[1], Vesna Stankov Jovanović[1]

Abstract: A set of "host" compounds, highly suluble in water, were prepared by the reaction of β-cyclodextrin with propylene oxide in NaOH solution. The reactant ratio was varied in order to examine the difference in the substitution degree of the obtained derivatives. The structure was determined by the ^{1}H-NMR spectra. The average degree of substitution was determined by integration of the corresponding NMR signals of the methyl group, which is part of the hydroxypropyl group and the signal from the proton attached to anomeric carbon of the β-cyclodextrin. The solubility of four different pesticides, very poorly soluble in water, was measured in water and in aqueous solution of the hydroxypropyl substituted cycloheptaamylose by ultraviolet spectrophotometry. Obtained results showed that the aqueous solution of hydroxypropyl-β-cyclodextrin was powerful solubilizer of investigated pesticides due to formation of stable inclusion complexes.

Keywords: β-cyclodextrin, epoxides, pesticides, water solubility

Introduction

β-Cyclodextrin (BCD) is a cyclic oligomer of 7 glucose residues. These glucose residues are arranged in a circle with a toroidal shape in which all the primary hydroxy groups are on the narrower base and secondary hydroxy groups are on the wider base of the toroid. This arrangement makes the cyclodextrin exterior decidedly hydrophilic. The secondary hydroxy groups can, however, interact via hydrogen bonding to stabilize the crystalline lattice. This reduces to a large extent the solubility of cyclodextrins in water. Most importantly, the interior of the cyclodextrin cone is hydrophobic and the result of this architecture is a lipoidal microenvironment which can solubilize non-polar compounds. In order to increase the water solubility of the cyclodextrins and their complexes, a great number of randomly substituted derivatives have

[1]University of Niš, Faculty of Sciences and Mathematics, Višegradska 33, 18 000 Niš, Serbia (peca@pmf.ni.ac.rs)
[2]University of Niš, Faculty of Medicine, Bulevar dr Zorana Djindjica 81, 18 000 Niš, Serbia

been prepared by the reaction of BCD with different reagents. In this way the hydrophobic cavity is extended, a greater surface area for complexation is provided and aqueous solubility of cyclodextrin is encreased because of the absence of crystalinity. The solubility and bioavailability of the active nonpolar compounds is consequently increased.

As with other nucleofiles, cyclodextrins, under basic conditions can be condensed with epoxides (Delgado et al. 1997). The opening of the epoxy ring occurs by nucleophilic attack of the alkoxy anion on the least substituted carbon atom. This mechanism can involve each of the three hydroxyl groups of BCD. Hydroxypropyl-β-cyclodextrin (HPBCD), the condensation product of the reaction of cyclodextrin with propylene oxide (PO), is a commercial derivative that has been widely used in the formation of host-guest complexes. The HPBCD samples can be characterized by the degree of substitution (DS, the average number of substituents on a cyclodextrin molecule). The acceptable range of DS of HPBCD is between 2.8 and 10.5 since HPBCDs with lower DS (DS<2.5) have poorer solubility than BCD itself.

The application of pesticides has become inevitable to protect crop plants from pests and diseases. Some hydrophobic organic pesticides have limitations for extensive application due to their low water solubilities and difficulty of their removal from soil. Agents such as organic cosolvents and surfactants have been considered for improving solubility of organic pesticides but both have some disadvantages for such applications. As an alternative, cyclodextrins may have potential for use as solubility enhancement agents for hydophobic organic pesticides (Lezcano et al., 2002) and their application in remediation of contaminated soil and groundwater (Mamba et al., 2007).

The objective of this paper is to study the the reaction of the BCD with PO, to isolate and purify three (**1**-**3**) cyclodextrin derivatives with different DS and to evaluate their ability to increase the aqueous solubility of four selected organic pesticides, so as to find a new method for improving the application of hydrophobic pesticides and enhancing their removal from the environment.

Materials and methods

The active components of the "dimethoate", "simazine", "linuron" and "thiram" (generic names of the pesticides) were obtained from Župa (Serbia) and were used without further purification. BCD was purchased from Merck (Germany) and was dried in vacuo till the constant weight prior to use. Propylene oxide, solvents and other chemicals used for the synthesis and analysis were of analytical grades purchased from Sigma-Aldrich.

The dialysis tubing was from Spectra-Por (MWCO 1000) with 28.6 mm diameter. ^{1}H NMR spectra were obtained with deuterated dimethyl sulfoxide (DMSO-d_6) as solvent at 298 K (10 mg of sample dissolved in 0.8 mL of deuterated solvent) and referenced to the TMS signal using a Gemini-200 spectrometer operating at 199,98 MHz for ^{1}H. Ultraviolet spectra were recorded using a Perkin-Elmer Lambda 15 spectrophotometer.

The set of "host" molecules (**1-3**), (2-hydroxypropyl) β-cyclodextrines with different DS, was obtained by the reaction of BCD with PO in aqueous NaOH solution with different molar ratios of reactants. Shortly, BCD was added to a 3M solution of sodium hydroxide and stirred at room temperature until complete dissolution. PO was added dropwise over 2 h and the mixture was stirred at room temperature overnight. The reaction mixture was neutralized with concentrated HCl, the solution was dialysed for 4 h using a 15 cm dialysis tube and evaporated under a reduced pressure. The solid obtained was treated with ethanol, stirred for 24 h and filtrated. The precipitate was dried under vacuum to obtain white solid.

The generator column approach was used to determine the solubilization of the pesticides by BCD and HPBCDs with different DS (Luo et al., 2003). The generator column used was a 25 cm long glass chromatography column packed with prewashed quartz sands coated with excess pesticides (0.01-0.1 g). The column was plugged with glass wool at both ends. 10 mL of distilled water or 10 mL of solutions containing different concentrations of BCD or HPBCDs were passed through this column, and a fraction of the effluent was immediately analyzed for solute concentration. The concentration of all samples was measured by UV spectrophotometry at the wavelength at which absorbance was maximum. The wavelengths used for UV detection were 192 nm for dimethoate, 221 nm for simazine, 246 nm for linuron and 207 nm for thiram. The effects of complex formation on the UV spectra of the pesticides were negligible within the range of experimental conditions.

Results and discussion

To ensure batch-to-batch consistency in production, all the reaction parameters were strictly controlled for the condensation of BCD with PO. Figure 1. presents the reaction and the products obtained.

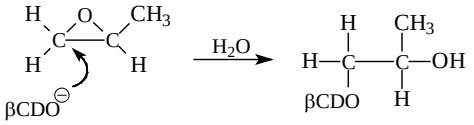

Figure 1: Reaction of β-cyclodextrin with propylene oxide.

The main byproduct formed during the reaction is propylene glycol but as the reaction proceeds, propylene glycol accumulates in the crude and can further react with propylene oxide to yield propylene glycol oligomers. In the same way, the 2-hydroxypropyl moieties of the formed HPBCDs can further react and generate oligomer-like side chains. The dialysis was used to purify the obtained compounds followed by the solid-liquid extraction with ethanol, in which only glycols are soluble. Kinetics of elimination of NaCl was measured by potentiometric control of chloride anion. After 4h of dialysis the concentration of chloride was less than 0.1%.

In this way three derivatives of BCD were obtained (**1-3**), with the structures shown and with different DS depending on the relation between the BCD, NaOH and oxirane. The ^{1}H-NMR spectra of HPBCDs are complicated by extensive overlap of the proton frequencies, only the anomeric signals (H1, 5.0-5.5 ppm) and the methyl frequencies (CH_3, 1.1-1-2 ppm) are clearly separated from the remaining protons. So, the average DS is calculated from the ratio between the signal from the 3 protons of the methyl group and the signal from the proton attached to the carbon C1. The average DS, yield and water solubility of HPBCDs are shown in Table 1.

Table 1. Analysis of HPBCDs with different degree of substitution

No.	Molar ratio CD/oxirane/Na(OH)	Yield (%)	DS	Solubility (g dm^{-3})
1	1/5/10	55	5.8	196
2	1/7/10	62	6.5	228
3	1/10/15	47	4.9	241

The aqueous solubility enhancement of BCD and HPBCDs on investigated pesticides were obtained by ploting the measured data according the equation:

$$S_t=S_o(1+K_sC_o)$$

where S_t is the aqueous phase concentration of pesticides with BCD or cyclodextrin derivatives, S_o is the concentration of pesticides without "host" molecules, C_o is the initial concentration of BCD and HPBCDs, and K_s is the stability constant of inclusion complexes for pesticides with BCD or HPBCD derivatives with different DS. We assume that the concentration of the "host" molecules is not depleted to an appreciable extent by complexing with pesticides. The relative aqueous phase concentrations (S_t/S_o) of the pesticides are plotted against the concentration of different "host" compounds.

Within the range of three β-cyclodextrin derivatives with different DS (**1-3**) used, the solubility of all four pesticides in solutions are linearly increased with

their concentrations progress. The data plot as a straight line with a slope rate less than 1, may be ascribed to the formation of 1:1 inclusion complexes in solution. The solubilization coefficients of BCD and HPBCD derivatives with different DS (KS_{CD}, KS_1, KS_2 and KS_3) on the four selected pesticides, respectively and some physical parameters are listed in Table 2.

Inspection of Table 2. reveals that the order of solubilization effects of BCD and all HPBCDs on the four pesticides is the same and it is dimethoate>simazine>linuron>thiram. The solubilization effects of HPBCDs on the pesticides are much more significant than that of the BCD. The limited water solubility of BCD (18.5 g dm^{-3}) itself was considered in an attempt to explain the phenomenon. On the contrary, HPBCDs are very water soluble, and their inclusion complexes with pesticides are therefore highly soluble.

Table 2. The solubilization coefficients of four pesticides and their selected physical parameters

	Dimethoate	**Simazine**	**Linuron**	**Thiram**
KS_{CD}	0.561	0.341	0.325	0.266
KS_1	0.935	0.876	0.810	0.755
KS_2	0.958	0.881	0.814	0.742
KS_3	0.982	0.885	0.829	0.783
MV (nm^3)	0.293	0.255	0.295	0.299
Solubility (mg dm^{-3})	39.8	6.0	77.0	8.4
log K_{ow}	4.56	4.74	4.29	3.77

The prerequisite for solutes to fit completely in the cavity of cyclodextrin and form an inclusion complex is that the molecule must be of appropriate shape and size. The size of BCD cavity is reported to be 0.346 nm^{-3}. Results in Table 2. show that the molecular volume (MV) of the selected pesticides, calculated with Sci Finder programe, are all smaller than BCD cavity volume. Hence, they can enter into the respective cavities of BCD or HPBCD and form inclusion complexes. The octanol/water partition coefficients (K_{ow}) have an important impact on the stability of the inclusion complexes. Inspection of Table 2. reveals that the selected pesticides have a large $\log K_{ow}$. Therefore, they can form stable inclusion complexes with cyclodextrin derivatives.

Pesticide pollution in the environment has caused increasing concern among the public. The increased solubilization of hydrophobic organic pesticides by HPBCD may leave the toxic substances more readily available for degradation by micro-organisms, and therefore reduce their residue in soils.

Thus HPBCDs are suggested for chemical and biological remediation enhancement techniques. However, whether they have adverse effects on bioavailability of pesticides needs to be evaluated.

Conclusion

The difference in the substitution degree of the obtained HPBCDs depends on the molar ratio of the reactants. The results showed that the effective solubility of the investigated pesticides in aqueous molecular receptor solutions was substantially increased. In comparision to cosolvents and surfactants as additives in the pesticide solutions formulations, cyclodextrin derivatives have many advantages. Most important, they are water soluble, nontoxic and biodegradable, thus posing no hazard to the ecosystem. Due to the fact that the cost of HPBCD is declining, price on Chinese market is now 1-10 $ per kg (depending on the ordering amount), it can be potentionaly useful for improving the application of hydrophobic organic pesticides and enhancing their removal from the environment.

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NOVEL TRENDS IN APPLICATION AND PRETREATMENT OF LIGNOCELLULOSIC AGRICULTURAL WASTE

Valentina Nikolić[1], Marijana Simić[1], Slađana Žilić[1], Danka Milovanović[1], Beka Sarić[1], Marko Vasić[1]

Abstract: Lignocellulosic biomass represents the most abundant renewable material in the world, whereas agricultural residues, including those from maize cultivation, comprise a significant fraction of the total plant waste that can be repurposed for various applications. Lignocellulosic feedstocks are non-edible and consist mainly of: cellulose, hemicellulose, and lignin, along with extractive compounds. Pretreatment is required to separate the lignocellulosic biomass into its constituents for efficient utilization. Even after extensive research and development of numerous techniques, pretreatment remains one of the most expensive phases in converting lignocellulosic biomass into biobased products.

Keywords: lignocellulosic biomass, agricultural waste, application, pretreatment.

Introduction

In the last few decades, lignocellulosic biomass has emerged as an increasingly popular for the manufacturing of products with added value. Globally, biomass resources are easily accessible as residual wastes from industrial and agricultural sources. Lignocellulosic biomass may provide numerous possibilities for producing environmentally friendly products (Figure 1), such as biofuels, biochemical, bioplastics and biocomposites for application in the biomedical, pharmaceutical, cosmetics, and other specialty material sectors (Okolie et al., 2021).

Considering lignocellulosic biomass is composed mainly of cellulose, hemicellulose, and lignin and is a component of plant cell walls, it is the prevailing type of biomass in the biosphere (Bayer et al., 2007). Lignocellulosic raw materials can be divided into several groups: 1) agricultural residues (waste from sugar cane, maize, wheat, rice and barley straw, rice husks, olive pits, cotton stalk, etc.); 2) forestry biomass (e.g. wood chips, wood logs, bark,

[1]Maize research Institute, Zemun Polje, Slobodana Bajića 1, Belgrade-Zemun, Serbia (valentinas@mrizp.rs)

sawdust, etc.) originating from both hardwood (aspen, poplar) and soft wood (spruce); 3) energy crops (switchgrass, timothy grass, elephant grass, poplar, willow) 4) cellulosic waste (old newspapers, used office paper, recycled paper pulp, etc.); 5) herbal biomass (alfalfa and other forage plants); and 6) municipal solid waste (Singh et al., 2020; Sanchez and Cardona, 2008).

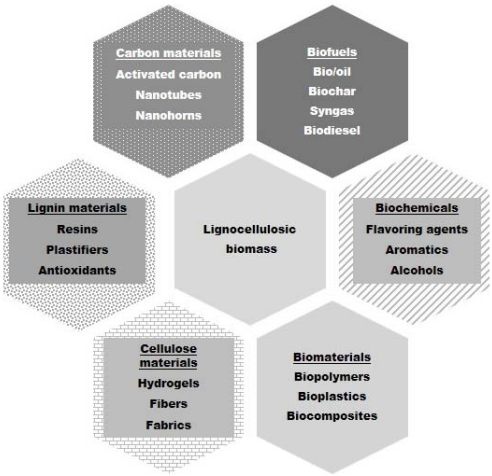

Figure 1. Possible applications of lignocellulosic biomass

Agricultural crop residues, including wheat straw, rice straw, corn cobs and straw, are regarded as significant and plentiful renewable biomass resources (Armah et al., 2020). Interest in maize as a renewable and biodegradable feedstock is increasing in the age of the global energy crisis brought on by the depletion of fossil fuel resources and the rise in environmental pollution. Agricultural waste from the cultivation of maize has a lot of potential for use in the production of bioethanol, highly absorbent water depollutants, bioplastics and other products (Semenčenko et al., 2009).

However, the major obstacle in lignocellulosic biomass conversion to biobased products is the pretreatment of the raw material. For enhancing the biodegradability and digestibility of crop residues, agricultural waste, and other lignocellulosic biomass, novel approaches for pretreatment of biomass may offer ecologically beneficial, economically viable, and sustainable options. The novel methods can be broadly categorized as physical, chemical, biological, physicochemical, and other cutting-edge green solvent-based pretreatment methods.

Composition of lignocellulosic biomass

The basic chemical components of lignocellulosic biomass which make up around 90% of the dry matter are cellulose (35–55 %), hemicellulose (20–40 %), and lignin (10–25 %), respectively.

Cellulose is the main component of the cell wall of higher plants (Okolie et al., 2021). Cellulose fibers ensure the strength of the plant material. When the grids of native cellulose are destroyed, on the primer with strong alkalis or by dissolving cellulose, there is a possibility of its regeneration. The chains of regenerated cellulose are parallel, but thermodynamically more stable than native cellulose (Semenčenko et al., 2011, Sanchez and Cardona 2008).

Hemicelluloses belong to the group of heteropolysaccharides. Softwood and hardwood hemicelluloses, although different in structure and composition hydrolyze to monomer components: glucose, mannose, galactose, xylose, arabinose and small amounts of rhamnose, glucuronic, methyl glucuronic and galacturonic acid. (Mojović et al., 2007). Hemicelluloses are mostly dissolved in alkalis so that they can be more easily hydrolyzed (Sanchez and Cardona, 2008)

Lignin is a very complex molecule composed of phenylpropane units. Wood has a high lignin content. Chemical bonds between lignin and hemicellulose and celluloses are ester, ether and glycosidic. Ether bonds make lignin extremely resistant to chemical and enzymatic decomposition, while biological decomposition is facilitated by many fungi and certain actinomycetes.

Extractives are among the main components of wood materials soluble in neutral organic solvents and water. They consist of a large number of lipophilic and hydrophilic components. Extractives can be classified into four groups: 1) terpenoids and steroids, 2) fats, and waxes, 3) phenolic compounds and 4) inorganic components (Mojović et al., 2007).

Pretreatment methods

Pretreatment procedures are carried out on biomass to overcome the biomaterial's initial resistance to conversion due to their complex physicochemical structure. The processes result in the breakdown of the biomass's components into cellulose, hemicellulose, and lignin (Figure 2). The porosity and surface area of the cellulosic moiety will increase as cellulose decomposes, while the crystallinity will decrease (Semenčenko et al., 2011).

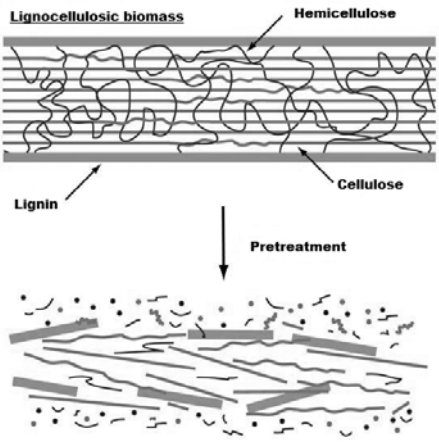

Figure 2. The effect of pretreatment on the lignocellulosic biomass structure

Agricultural waste and other lignocellulosic biomass are pretreated primarily to lower the amount of energy required for conversion, lower the cost, and produce sugars directly from the biomass (Areepak et al., 2022; Zhang et al., 2021). Physical, chemical, biological, physicochemical, and green solvent-based processes are some of the typical pretreatment methods (Awogbemi and Von Kallon, 2022). However, traditional physicochemical methods for removing lignin from lignocellulose feedstocks entail the use and synthesis of a variety of hazardous chemical compounds, some of which can hinder the process itself, as well as considerable energy consumption (Saha et al. 2016). The biological delignification of lignocellulose is an alternative process that is less expensive and safe for the environment. Although biological treatments have clear advantages over conventional ones, they also have certain drawbacks, such as taking longer and being less effective (Ćilerdžić et al., 2022). It is generally recognized that white-rot fungi and their ligninosomes, composed of peroxidases and laccases, are the most promising candidates for the biological pretreatment of lignocellulose. They differ substantially in terms of the method of depolymerizing lignocellulose, i.e., whether they degrade lignocellulose polymers concurrently or only lignin (Saha et al. 2016). According to Knežević et al. (2014), some species have already been confirmed as effective delignifiers of wheat straw, rice straw, oak sawdust, and oil palm wastes, respectively.

Conclusion

Current trends in research regarding the improvement of pretreatment and processing of lignocellulosic biomass, including agricultural residues are closely related to the nature and complex structure of biomaterials. Pretreatment is unavoidable due to the benefits it provides, although adding to the cost, infrastructure needs, labor demands, and energy consumption of the entire production process. Novel methods for pretreatment of biomass may provide environmentally advantageous, financially feasible, and sustainable solutions for improving the biodegradability and digestibility of crop residues, agricultural waste, and other lignocellulosic biomass. Analyzing the possible energy and economic impacts of the pre-treatment process is required to make efficient use of the raw material. Although biological treatments clearly outperform conventional ones, they also have some disadvantages, such as longer treatment times and lower efficacy. Mechanical pretreatment should be reduced, despite the fact that it is frequently necessary, while the chemical pretreatment should be used when processing wood. Using a mix of various approaches is the new strategy to get beyond the drawbacks of pretreatment methods that are carried out as a single operation.

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LENGTH-WEIGHT RELATIONSHIP OF NINE FISH SPECIES FROM GRUŽA RESERVOIR (CENTRAL SERBIA)

Nataša Kojadinović[1], Milena Radenković[1], Simona Đuretanović[1], Aleksandra Milošković[2], Marija Jakovljević[1], Tijana Veličković[1], Vladica Simić[1]

Abstract: Length–weight relationship give information on the condition and growth patterns of fish. This study reports length– weight relationships for *Abramis brama* (Linnaeus, 1758), *Alburnus alburnus* (Linnaeus, 1758), *Carassius auratus* (Linnaeus, 1758), *Cyprinus carpio* (Linnaeus, 1758), *Rutilus rutilus* (Linnaeus, 1758), *Silurus glanis* (Linnaeus, 1758), *Ameiurus nebulosus* (Le Sueur, 1819), *Perca fluviatlis* (Linnaeus, 1758) and *Sander lucioperca* (Linnaeus, 1758). Specimens were collected from 2007 to 2013 in Gruza Reservoir (Central Serbia). The b values in the LWRs of analyzyed fish varied between 2.274 and 3.213.

Keywords: freshwater fish, Central Serbia, length-weight, fishing ecology

Introduction

Length–weight relationships (LWRs) were originally used to provide information on the fish condition and to determine whether somatic growth was isometric or allometric (Le Cren, 1951; Ricker, 1975). LWRs are fundamental in fisheries research (Tsionki et al., 2021). In particular, thew can be used for the determination of weight and biomass from length data, as indications of the body condition and for comparisons of species–specific growth between regions and among seasons provided that the same sampling methodology is used (Bobori et al., 2010, Froese 2006., Petrakis & Stergiou, 1995).

Materials and methods

The Gruža Reservoir is situated in Central Serbia, near the city of Kragujevac, at an altitude of 269 m, with a surface area of 9.34 km^2 and a maximum depth of

[1]Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Kragujevac, Serbia
(natasa.kojadinovic@pmf.kg.ac.rs)
[2]Institute for Information Technologies Kragujevac, University of Kragujevac, Kragujevac, Serbia

31 m (Fig. 1). It was formed in 1985 to supply drinking water to the population of the region, and also for recreational purposes (Milošković, 2013).

The Gruža reservoir biotope is extremely favourable for the development of different hydrobiont communities and high fish fauna production (Marković, 2011).

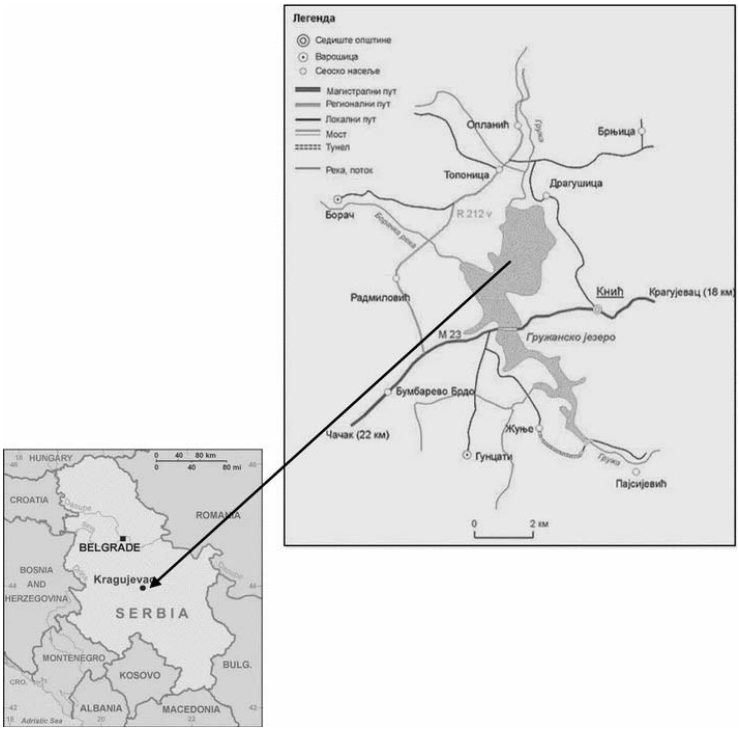

Figure 1. Location of the Gruža Reservoir near the city of Kragujevac, Central Serbia

Fishes were sampled from 2007 to 2013, using gillnets with different mesh sizes (from 10 to 120 mm) and electrofishing (Aquatech IG 1300). In the laboratory sampled specimens were identified to species according to Kottelat and Freyhof (2007) and Simonović (2001), then measured to the nearest 1 mm (total length, L) with a sliding caliper and weighed to the nearest 0.1 g (weight, W).

Length–to–weight relationship for total body weight was calculated using the equation W ¼ aLb, where W is the total weight (expressed in grams), L is the total length (expressed in centimetres), a is a coefficient related to body form and b is an exponent indicating isometric growth when equal to 3.

The parameters a and b were estimated by linear regression on the transformed equation: log(W) ¼ log(a) + b log(L) (Koutrakis and Tsikliras, 2002)

Results and discussion

In this study, 1752 specimens of fish belonging to nine species from five families were sampled: *Abramis brama* (Linnaeus, 1758), *Alburnus alburnus* (Linnaeus, 1758), *Carassius auratus* (Linnaeus, 1758), *Cyprinus carpio* (Linnaeus, 1758), *Rutilus rutilus* (Linnaeus, 1758), *Silurus glanis* (Linnaeus, 1758), *Ameiurus nebulosus* (Le Sueur, 1819), *Perca fluviatilis* (Linnaeus, 1758) and *Sander lucioperca* (Linnaeus, 1758). Table 1 shows the sample size, minimum and maximum length, parameters a and b values and the coefficient of determination (r^2).

Table 1. Descriptive statistics and estimated parameters of length-weight relationships for nine species caught in Gruža Reservoir

Family	Species	n	Min TL	Max TL	a	b	SE (b)	r^2
Cyprinidae	*Abramis brama*	845	14.2	44.5	0.034	2.617	0.046	0.892
	Carassius auratus	542	10.5	43.0	0.016	2.992	0.720	0.872
	Cyprinus carpio	49	26.0	82.1	0.018	2.933	0.073	0.999
Leuciscidae	*Alburnus alburnus*	73	13.1	17.2	0.059	2.274	0.408	0.840
	Rutilus rutilus	74	12.5	28.5	0.007	3.213	0.084	0.976
Siluridae	*Silurus glanis*	10	49.6	188.4	0.005	3.044	0.073	0.998
Ichtaluridae	*Ameiurus nebulosus*	21	11.5	26.8	0.006	3.200	0.104	0.990
Percidae	*Perca fluviatilis*	32	10.5	30.0	0.015	2.903	0.275	0.888
	Sander lucioperca	106	22.0	66.2	0.004	3.182	0.109	0.944

N, sample size; Min TL, minimum values of total length; Max TL, maximum values of total length; a and b, parameters of the equation; SE(b), standard error of b; r^2, coefficient of determination.

The slope b value of length–weight relationships provides useful information on fish growth. It shows isometric growth when b=3, while it indicates positive allometry when b>3, and negative allometry when b<3 (Tarkan et al., 2006). Positive or negative allometry indicates a rounder or slimmer body, respectively, whereas isometric growth shows that the body grows in the same proportion in all dimensions (Jobling, 2008).

The b values varied from 2.274 for *A. alburnus* to 3.213 for *R. rutilus*. Similar results for *R. rutilus* (b 3.270) were found by Tsoumani et al. (2013). In Gruža Reservoir five of the nine analysed species showed negative allometric growth (*A. brama*, *A. alburnus*, *C. auratus*, *C. carpio* and *P. fluviatilis*), and in this case ones become more elongated as it increases in length. Earlier researches reported that negative allometric growth pattern with b value less than 3 exist in *A. brama* (Guettaf et al., 2019) and *A. alburnus* (Lujić et al., 2013). Amdreu-Soler (2006) corroborate the positive allometric growth for *S. lucioperca* reported in our study.

On the other hand, Marinovic et al. (2016), reported that *C. auratus* from the Gruža Reservoir displayed isometric growth which is not in conformity with the current study. This may be possibly due to several factors such as the number of specimens examined, food availability, season and sex

Conclusion

The present study is the first attempt to provide information about lenght-weight relationships of fish species in Serbia, and could give useful insight for management and conservation of these species

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APPLICATION OF QPCR FOR PLUM POX VIRUS DETECTION DURING CRYOTHERAPY

Darko Jevremović, Bojana Vasilijević, Tatjana Anđelić, Tatjana Vujović

Abstract: For the purpose of removing viruses from infected plant material, cryotherapy is a novel application of the plant cryopreservation technique. The use of various cryotherapy procedures and treatments necessitates the examination of cryo-treated material for the presence of the targeted pathogen in order to gauge the effectiveness of the therapy. In our study, we evaluated the efficiency of reverse transcription-polymerase chain reaction (RT-PCR) and qPCR (quantitative PCR) methods for the detection of plum pox virus (PPV) in cryo-treated material of plums 'Belošljiva' and 'Crvena Ranka'. A qRCR assay showed higher sensitivity in comparison to conventional RT-PCR.

Keywords: plum pox virus, qPCR, cryotherapy, efficiency

Introduction

A safety measure against the unintentional loss of plant germplasm collections, the latest biotechnology-based conservation strategies, including various *in vitro* procedures, complement *ex situ* conservation techniques. Cryopreservation, or the storage of plant material at extremely low temperatures (-196°C), has emerged as a crucial method for the long-term preservation of plant germplasm. Recently, it has been shown that cryopreservation can be employed for other uses than germplasm conservation – for eradicating viruses. Cryotherapy is a cutting-edge method of removing pathogens from infected plant material. Once the methodology for the specified genotype has been established, the technique requires only the most basic tools found in a tissue culture laboratory. The main difficulty in applying cryotherapy is the vastly varied responses to the treatment between genotypes of the same species. For a dozen species, a number of procedures using various cryotherapy techniques have been documented.

The most devastating viral disease in stone fruits, including plum, is plum pox virus (PPV) that is present in Serbia since 1930ies. Other known viruses infecting stone fruits were very rarely detected in plums in the country. In the

[1]Fruit Research Institute, Kralja Petra I nr. 9, Čačak, Serbia (darkoj@ftn.kg.ac.rs)

past decade, research on PPV in Serbia has brought new knowledge on PPV distribution, genetic diversity, and epidemiology (Jevremović, 2013). According to the estimates, about 70% of the plum trees are infected with three major PPV strains (PPV-M, -D, and -Rec). PPV represents the main threat to the production and existence of plum genotypes in an open field. Cryotherapy, as method for PPV eradication from stone fruit species is an old idea, but not much investigated and utilized in the practice. An important segment in this process is the analysis of the regenerated cryopreserved material. Highly sensitive assay is required to detect PPV, even in a very low concentration due to the suppression with the applied cryotherapy method.

In this paper we presented the results of the application of quantitative Polymerase Chain Reaction (qPCR, Real-time PCR) for PPV detection in cryopreserved plums.

Materials and methods

The initial material for this experiment consisted of two PPV-infected autochthonous plums 'Belošljiva' and 'Crvena Ranka'. Aseptic cultures of these two plum cultivars were established on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) as described in detail by Jevremović et al. (2022). Prior cryotherapy, apical shoot tips (1 mm large) and axillary buds were dissected from the shoots and analyzed by qPCR to confirm the presence of PPV. Cryotherapy was performed on plum shoot tips (aprox. 1.5 mm long) from 4-week-old plantlets growing on MS media using aluminum cryo-plates according to D- and V-cryo-plate methods (Jevremović et al., 2022).

A total of 111 pool samples of *in vitro* shoots (average 10 plants per sample, in total about 1100 plants) of the plums 'Belošljiva' and 'Crvena Ranka' were tested (65 samples of 'Belošljiva' and 46 samples of 'Crvena Ranka'). *In vitro* shoots regenerated from control and cryopreserved explants were continuously tested during multiplication for the presence of PPV.

At the first stage (the first two subcultures after regrowth), all samples were tested with conventional RT-PCR analysis. To evaluate these results and obtain the highest efficiency of detection, all samples were further tested using qPCR.

Total nucleic acids (TNA) were isolated from 0.2 g fresh *in vitro* shoots with 2% CTAB buffer according to the protocol of Li et al. (2008). Extracted RNA was used for two-step reverse-transcription (RT) analysis. Reverse transcription (RT) reactions were performed in two steps with Maxima reverse transcriptase (ThermoScientific, USA). Obtained cDNA was subjected to PCR reaction with

P1/P2 universal primers (Wetzel et al., 1991). RT and PCR reactions were performed in TPersonal thermal cycler (Biometra, Germany). PCR products were analysed by 1.5% agarose gel electrophoresis, stained with ethidium bromide and visualized by Gel Doc EZ System (Biorad Laboratories, USA). The presence of an expected fragment of 243 bp was considered as a positive reaction.

Samples were further tested using primers and TaqMan probes (Olmos et al., 2005). The reaction mixture (20 µl) consisted of: 2 × TaqMan Universal PCR Master Mix (Applied Biosystems, USA), 6.25 U 1 × MultiScribe RT (Applied Biosystems, USA), 10U RNase Inhibitor Mix (Applied Biosystems, USA), 1 µM P241 primer, 0.5 µM each of P316D and P316M primers, 200 nM TaqMan PPV-DM probe and 5 µl RNA template. The reaction was performed with the following thermocycling conditions: 15 min at 48°C 10 min at 95°C, and 40 cycles of 15 s at 95°C and 60 s at 60°C. Data acquisition and analysis were conducted in a StepOnePlus™ Real-Time PCR System (Applied Bio-systems, USA) and StepOne™ v2.3 Software package (Life technologies, USA).

Results and discussion

Using RT-PCR, the plum pox virus was detected in 67 out of 111 samples tested (60.36%). An expected 243 bp fragment was obtained in 39 samples of plum 'Belošljiva' and 28 of plum 'Crvena Ranka' (Figures 1 and 2). In certain positive samples (Figure 1, lines 8, 9, 11, 12, 14-18; Figure 2, line 18), expected fragments were clear, and strong suggesting a relatively high virus concentration in the samples. In some samples, bands on the gel were very faint and barely visible (Figure 2, lines 5, 12, 14-17).

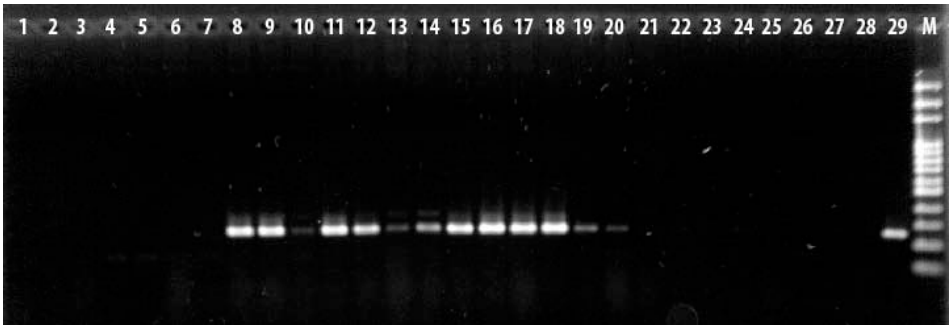

Figure 1. RT-PCR detection of plum pox virus in *in vitro* plants after cryotherapy (lines 1-28: analysed samples, line 29: positive control; line M: 100 bp DNA Ladder (Solis BioDyne, Estonia)

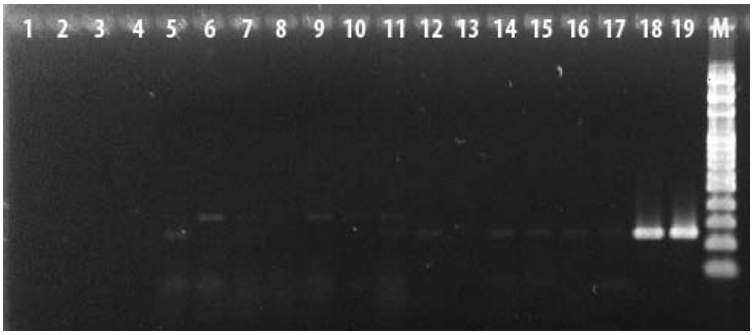

Figure 2. RT-PCR detection of plum pox virus in *in vitro* plants after cryotherapy (lines 1-18: analysed samples, line 19: positive control; line M: 100 bp DNA Ladder (Solis BioDyne, Estonia)

Using a qPCR assay, PPV was confirmed in 82 tested samples (73.87%). Conventional RT-PCR failed to detect PPV in 15 samples that were positive with qPCR (Figures 3 and 4). In control (non-cryo-treated) plants, PPV was detected with both assays.

Quantitative PCR proved to be a more reliable method for the analysis of *in vitro* plants after cryotherapy. Due to the low virus concentration in regenerated plants, a highly sensitive method is needed to evaluate the health status of the material and the success of cryotherapy. Cryotherapy has a significant impact on virus concentration in treated plants and can eradicate PPV in certain treatments and genotypes (Jevremović et al., 2022).

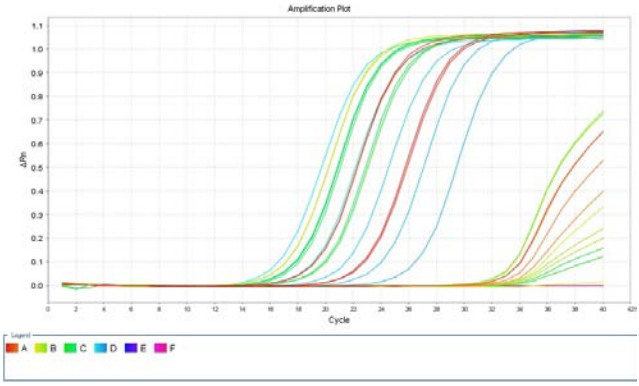

Figure 3. qPCR detection of plum pox virus in analyzed samples

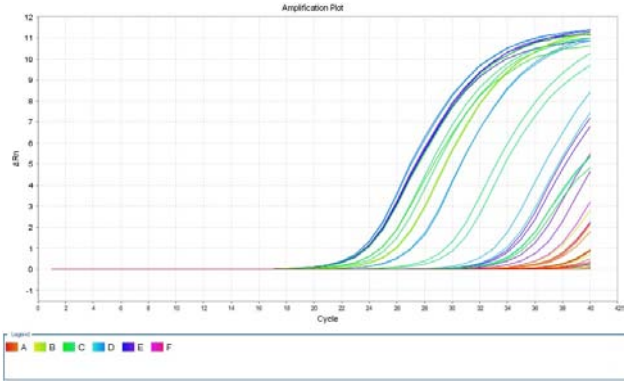

Figure 4. qPCR detection of plum pox virus in analyzed samples

In published studies on plum pox virus eradication with different methods (hemotherapy, thermotherapy, cryotherapy, and their combinations) ELISA (Enzyme-Linked ImmuneSorbent Assay) and RT-PCR methods were performed (Brison et al., 1997; Manganaris et al. 2003; Paunović et al., 2007; Polak and Hauptmanova 2009). Since its discovery, PCR has become the gold standard for the molecular detection of a large number of plant pathogens. Real-time PCR provide a more rapid, sensitive and reliable diagnosis of PPV (Olmos et al., 2006). For the first time, two molecular methods were evaluated in our study to gain access to the health status of cryopreserved plum material. Based on our results, qPCR should be used in the analysis of the plant material on the presence of PPV during cryotherapy.

Conclusion

The findings of our investigation supported the excellent specificity and sensitivity of the qPCR technique for the detection of PPV in *in vitro* shoots of the plum cultivars 'Belošljiva' and 'Crvena Ranka'. Higher sensitivity is one of the characteristics of qPCR in contrast to PCR.

Acknowledgement

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STATE OF SOIL FERTILITY IN THE AREA OF THE POŽAREVAC CITY

Vladanka Stupar[1], Markola Saulić[1], Milica Blažić[1], Zlata Živković[1], Darko Stojićević[1], Marko Stokić[2], Bojan Stević[2]

Abstract: : Soil analysis provides information about the quality of the soil and the input of nutrients that are needed to make the soil better and obtain a high crop yield. The research in this paper included the analysis of fertility parametars of soil in wider territory of the Požarevac city during 2017 and 2022 year. Soil analysis shows that average soil acidity, i.e. its pH value of the aqueous soil solution (pH - H2O), in 2022 was reduced by 0.28 compared to 2017. On other hand, the substitution acidity (pH - KCl) was reduced by 0.49 after 5 years. The results for CaCO3 show that the soils are generally carbonate-free or weakly carbonated , well supplied with humus and the percentage of contetnt of N decreased by 21.91% in the five-year period. Low content of easily accessible phosphorus was identified in both year in over 50 percentage of the soil samples while mostly soil samples are well supplied with easily accessible potassium.

Keywords: soil, pH, fertility parameters, Požarevac city

Introduction

From an agronomic aspect soil present a basic resource of agricultural production and its fertility is a dynamic natural property which can change under the influence of natural and human induced factorsthis depletion can be due to excessively intense cultivation and inadequate soil management (Amara et al., 2017). Widespread deficiencies of N, P, K, S, Zn, Fe, B etc are so intense and severe that visual symptoms are very often observed in major crops In intensively cultivated soils are being depleted with available nutrients especially secondary and micronutrients. For that reason, evaluation of fertility status of the soils of an area or a region is one of the most important aspect in the context of sustainable agriculture (Singh and Mishra, 2012). Also, it is one of the measures aimed at protecting and preserving the chemical and biological properties of agricultural soil and ensuring the proper use of mineral and organic fertilizers is the systematic control of soil fertility. Soil analysis is usually carried out to check in for soil quality, nutrient content, changes in

[1] Academy of Applied Techical Studies Belgrade, Colleage of Applied Engineering Sciences, Nemanjina 2, Požarevac, Serbia (Vladanka Stupar vstupar@atssb.edu.rs)
[2] Agricultural Advisory and Expert Service Pozarevac d.o.o., Dunavska 91, Požarevac, Serbia

various parameters of soil. It gives all the information about nutrient inputs required to make the soil better and as well as soil quality in order to obtain high yields (Bhatia et al., 2021). The basic parameters of soil fertility include active and substitution acidity, content of carbonates, humus, easily accessible forms of phosphorus and potassium and the content of total nitrogen.

This paper compares the state of fertility based on agrochemical analyzes in the wider territory of city Požarevac with city municipality Kostolac and 25 rural settlements) during 2017 and 2022.

Materials and methods

The territory of today's city covers an area of 47,100 ha, of which as much as 39,240 ha (83.3% of the total territory) is arable land. In addition to mineral wealth (coal) and thermal energy plant (TE-KO Kostolac), the land is one of the most valuable assets of this region because three rivers flow through it: Danube, Velika Morava and Mlava. The presence of different geomorphological units, climatic conditions and the presence of surface and groundwater had a visible impact on the formation different soil types and subtypes: Chernozems (leached, marshy, degraded), Vertisols, Vertisol allluvial, Vertisol in podzolization, Eutric Cambisols, Eutric Cambisols in podzolazition, riparian black soil (Humis Gleysols), ponds and swamps (Mrvić et al., 2016).

Table 1. Multi-year average (1990-2020) of mean monthly, annual and extreme values for Veliko Gradište (44° 45′ 14.4″ N, 21° 30′ 29.4″ E, altitude 68 m)

AIR TEMPERATURE (°C)													
Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Average
Average	0.5	2.2	6.8	12.3	17.1	20.8	22.6	22.2	17.2	11.9	6.9	1.7	11.9
Absolute maximum	17.5	22.2	26.4	31.6	35.3	38.6	43.6	40.6	36.7	31.1	25.5	18.0	43.6
Absolute minimum	-22.2	-23.8	-19.6	-7.9	-1.1	3.4	5.9	5.3	-1.3	-8.0	-10.3	-19.4	-23.8
RAINFALL (mm)													Annual sum
Average monthly sum	44.6	41.6	41.5	56.5	73.2	76.0	76.3	52.3	58.9	54.3	45.4	49.2	669.8

In 2017, 330 soil samples from individual producers were examined. Farmers recognized the importance of systematic soil control, so in 2022, twice as many

samples were examined, 750. The samples were taken from a depth of 0-30 cm. Agrochemical soil analyzes during both years (2017 and 2022) were carried out in the laboratory of the Agricultural, Expert and Advisory Service of Požarevac, according to the following methods: determination of pH value (potentiometric) - method SRPS ISO 10390: 2007;–determination of carbonate content (volumetric) - method SRPS ISO 10693:2005;–determination of humus content by the Kotzmann method (volumetric) - VDM 01;–determination of easily accessible potassium by AL method according to EngerRiehm (flamephotometric) - VDM 02 and determination of easily accessible phosphorus by AL method according to EngerRiehm (spectophotometrically) - VDM 03.

Statistical data processing (average value, standard deviation and coefficient of variance) was performed using the 2016 Excel program.

Data on multi-year climate parameters of the research site (1990-2020) were collected at the meteorological station Veliko Gradište (44° 45 14.4 ″ N, 21° 30′ 29.4″ E, altitude 68 m) (www.hidmet.gov.rs), and are shown in Table 1.

Results and discussion

Soil analysis in 2017 and 2022 year shows that average soil acidity, i.e. its pH value of the aqueous soil solution (pH - H_2O), in 2022 was reduced by 0.28 compared to 2017 (table 2). On other hand, the substitution acidity (pH - KCl) was reduced by 0.49 after 5 years. This results cannot be fully attributed to further soil acidification processes, taking into account the double coverage of samples in 2022. However, worrying data shows the acidity of the solid phase of the soil in 2022. More than a third of the examined samples (37.73%) were in the class of acidic or highly acidic soil (pH 4.5-5.5 and pH <4.5) (table 3). In 2017, 18.48% belonged to these land classes.

Table 2. Average values, absolute minimum, absolute maximum, deviation standard and coefficient of variance for surveyed soil fertility elements

Elements of fertility	pH (H_2O)	pH (KCl)	$CaCO_3$ (%)	Hummus (%)	N (%)	P_2O_5 (mg 100 g^{-1})	K_2O (mg 100 g^{-1})
2017							
$\overline{X}$	7.01	6.32	0.60	3.79	0.19	12.55	25.57
MIN	5.15	4.15	0.00	2.00	0.10	1.09	8.63
MAX	8.40	8.00	5.78	8.12	0.41	40.00	40.00
6	0.75	0.93	1.17	0.95	0.05	10.58	8.04
CV	0.11	0.15	1.95	0.25	0.25	0.84	0.31

2022							
$\overline{X}$	6.73	5.83	1.05	3.30	0.16	12.66	27.20
MIN	4.79	3.96	0.10	1.70	0.09	2.32	5.00
MAX	8.46	7.64	6.08	6.94	0.35	40.00	40.00
6	0.82	0.90	1.28	0.70	0.03	11.14	9.22
CV	0.12	0.15	1.22	0.21	0.21	0.88	0.34

Soil pH, as is the main factor that affects many biological, chemical and physical soil properties, has a direct or indirect effect on nutrient availability, with the ideal range generally being between 6.5 and 7.3 (Chaouqi et al., 2018).

Table 3. Grouping of soils according to the values of examined fertility elements

Elements of fertility	Distribution	2017		2022	
		n	%	n	%
pH (H_2O)	> 7.2	119	36.06	272	36.27
	6.5-7.2	115	34.85	179	23.87
	5.5-6.5	92	27.88	235	31.33
	4.5-5.5	4	1.21	64	8.53
	< 4.5	0	0.00	0	0.00
pH (KCl)	> 7.2	90	27.28	60	8.00
	6.5-7.2	33	10.00	133	17.73
	5.5-6.5	146	44.24.	275	36.67
	4.5-5.5	54	16.36	224	29.87
	< 4.5	7	2.12	58	7.73
$CaCO_3$ (%)	> 10.0	0	0.00	0	0.00
	5.0-10.0	6	1.82	11	1.46
	0.1-5.0	112	33.94	149	19.87
	< 0.1	212	64.24	590	78.67
Hummus (%)	> 10	0	0.00	0	0.00
	5-10	26	7.88	12	1.60
	3-5	240	72.73	497	66.27
	1-3.0	64	19.39	241	32.13
	< 1	0	0.00	0	0.00
N (%)	> 0.3	11	3.33	1	0.13
	0.2-0.3	111	33.64	112	14.93
	0.1-0.2	208	63.03	625	83.34
	0.06-0.1	0	0.00	12	1.60
	0.03-0.06	0	0.00	0	0.00

P_2O_5 (mg 100 g^{-1})	>20	59	17.88	134	17.87
	10-20	77	23.33	170	22.67
	<10	194	58.79	446	59.46
K_2O (mg 100 g^{-1})	>20	240	72.73	542	72.26
	10-20	86	26.06	200	26.67
	<10	4	1.21	8	1.07
N		330		750	

Severe acidification can cause irreversible dissolution of clay minerals and reduction of cation exchange capacity, followed by structural deterioration (Goulding, 2016). In alkaline or slightly acidic soil biodegradation increases, while an acidic environment is a limitation biodegradation (Pawar, 2015). The optimal soil pH value for microbial activity is ranges from 5.5 to 8.8 (Pietri and Brookes, 2008). Therefore, special attention should be paid to ameliorative measures of calcification of these soils (Stokić et al., 2022) and the choice of mineral fertilizers with a neutral physiological reaction.

The results for $CaCO_3$ show that the soil of the city of Požarevac is generally carbonate-free or weakly carbonated (2022 – 98.54%; 2017 – 98.14%), which is a consequence of the absence of $CaCO_3$ in the parent substrate.

Based on the results in Table 3, it can be concluded that the tested soils are mostly well supplied with humus. Weak humus soils in 2022 make up 32.13%, and in 2017, 19.39%. These soils should be given more attention in terms of organic matter intake, either by adding manure or the increasingly popular pelleted manure, by plowing the crop residues and by growing other crops for green fertilization. Humus in the soil is not only an important source of nutrients for higher plants, but also a food source for soil microorganisms (Belić et al., 2014) and is directly related to the production of C, N and S in the soil. Nitrogen in the soil mostly originates from organic matter and is directly related to the content of humus in the soil. For this reason, the results for the N content in the soil indicate a good (2022 - 83.34%; 2017 - 63.03) and rich supply (2022 - 15.06%; 2017 - 36.97%). However, it can be observed that the percentage of soil rich in N decreased by 21.91% in the five-year period.

The low content of easily accessible phosphorus was identified in 58.79% of samples in 2017 and 59.46% in 2022. Weak supply of phosphorus is conditioned by its absence in the geological substrate, poor management of reclamation fertilizers, the presence of clayey soils and an acidic environment. Therefore, on the basis of agrochemical analyses, special attention should be paid to the intake of phosphorus fertilizers in stock, i.e. ameliorative fertilizer.

When looking at the data on the content of easily accessible potassium in the soil in both compared years, it can be concluded that they are mostly well supplied with this nutrient (2022 - 72.26%; 2017 - 72.73%). The high content of readily available potassium can be a consequence of pedogenesis, because most of the soils of the Braničevo district were formed on a parent substrate rich in potassium (Vasin et al., 2004), as well as the predominance of mica and potassium-rich feldspar minerals (Stokić et. al., 2022).

Conclusion

Farmers of wider territory of the Požarevac have recognized the importance of systematic soil control. In 2017, 330 soil samples were examined, while only 5 years later, twice as many samples (750) were sampled and examined. Comparing the samples, it is observed that the acidity is reduced after 5 years but attention should be paid to ameliorative measures of calcification of these soils and the choice of mineral fertilizers with a neutral physiological reaction. Although the soils were examined are mostly well supplied with humus itshould be given more attention in terms of organic matter intake, either by adding manure or the increasingly popular pelleted manure, by plowing the crop residues and by growing other crops for green fertilization. Low content of easily accessible phosphorus insoil samples show that soil has poor management of reclamation fertilizers. On the other hand, most soils are well supplied with easily accessible potassium.

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BIOINDICATION ASSESSMENT OF WATER, AIR AND SOIL QUALITY

Dragutin Đukić[1], Leka Mandić[1], Vesna Đurović[1], Marijana Pešaković[2], Monika Stojanova[3]

Abstract: Various bioindicative organisms can be used for rapid assessment of environmental quality. For the bioindicative assessment of air quality lichens are most often used; primarily planktonic microorganisms as well as benthic organisms including macrophytes for water quality; microbiological, enzymatic, lichenological, zoological and phytoindicative methods for quality of soil.

Keywords: air, water, soil, bioindicators, quality

Introduction

Bioindicative research detects and determines ecologically significant natural and anthropogenic pollution based on the reactions of living organisms in the environment.

Biological indicators are characterized by properties that are characteristic of a system or process, on the basis of which a quantitative or qualitative assessment of change tendencies, determination or qualification of the state of ecological systems, processes and phenomena is carried out.

There are great possibilities of using living organisms in bioindicative research, especially indicator species, which are able to survive in a narrow interval of action of a certain factor, based on their morphological, anatomical, physiological, biochemical, genetic and other properties. With their presence, they are indicator of the existence of that factor in the environment. With the help of micro- and macro-organisms, enzymatic activity and genetic properties of the mentioned organisms, bioindicative studies of air, water and soil quality (Bioindication) are carried out - Đukić et al., 2013.

[1] University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia (lekamg@kg.ac.rs)
[2] Fruit Research Institute Čačak, Kralja Petra I 9, 32102 Čačak, Serbia
[3] University of Ss. Cyril and Methodius, Faculty of Agricultural Sciences and Food, Skopje, North Macedonia

Biological diagnosis of air

As is known, air is a mixture of certain gases, which are present everywhere on Earth in approximately equal parts by volume. Air pollution occurs when there are substances in the mixture in such quantities and for such a long time that they become a danger for humans, animals, plants or material goods (Đukić and Mandić, 2005; Đukić et al., 2022). Air pollution has a harmful effect on all living organisms, especially plants. Plants are best suited for detecting initial changes in air composition. Corresponding indices indicate the toxic effect of substances that pollute the air.

Many researchers have determined the importance of lichens in the bioindication of air quality (lichenindication) - Table 1. They have very specific properties because they react to changes in the composition of the atmosphere. Their biochemistry is different from other organisms, they are widely distributed on different types of substrates, starting with rocks, up to the bark and leaves of trees, and are exposed to direct action (Szwed et al., 2020).

It is possible to obtain completely reliable data on the level of air pollution with the lichens (Samsudin et al., 2012). Lichens are particularly sensitive to: sulphur and nitrogen oxides, hydrogen fluoride and chloride and heavy metals. Many lichens die at low levels of atmospheric pollution with these substances.

Table 1. Air quality scale based on the projected coverage of trees with lichens (Đukić et al., 2013)

Degree of coverage	Number of species	The number of lichens as dominant species	Degree of pollution
More than 50%	More than 5	More than 5	VI zone Very clean air
	3 – 5	More than 5	V zone Very clean air
	2 - 5	Less than 5	V zone Relatively clean air
20 – 50 %	More than 5	More than 5	
	More than 2	Less than 5	III zone Moderate pollution
< 20%	3 - 5	Less than 5	II zone Heavy pollution
	0 – 2	Less than 5	I zone Very heavy pollution

Assessment of air purity can also be done using plants. For example, gymnosperms are excellent indicators of atmospheric cleanliness. It is also possible to study mutations in the fibers of the anther filaments of tradescantia. It has been observed that with an increase in carbon monoxide and nitrogen oxides in the air, emitted by internal combustion engines, the color of the stamens changes from blue to pink. The consequences of disturbances in the individual development of plants can also be determined based on the frequency of finding morphological deviations (phenodeviants), the size of fluctuating asymmetry indicators (deviation from perfect bilateral and radial symmetry) by the method of analyzing complexly organized complex structures (Mandal, 2006). Significant changes are observed in some biochemical characteristics of plants, such as the content of water, chlorophyll, carotenoids, pH of the extract, content of phenol, ascorbic acid etc. (Uka et al., 2017). The levels of any deviations from the norm are minimal only under optimal conditions and increase under any stressful influences.

Biological diagnostics of water

It should be emphasized that biological research does not study water, but the water basin as a whole as a unique ecosystem. Stroganov (1983) defined water toxicology as the science of environmental toxicity of hydrobionts at all levels of life organization which studies all responses of hydrobionts to any pollution.

In order to assess the level of toxic water pollution with industrial or other wastewater, it is necessary to answer the following questions: is the water that reaches the water basin with wastewater toxic; what is the degree of its toxicity; at what distance from the source of pollution does the toxicity decrease to a minimum value?

All groups of organisms that inhabit water basins can be used for the biological indication of water quality: planktonic and benthic invertebrates, protozoa, algae, macrophytes, bacteria and fish (Đukić and Ristanović 2005; Li et al., 2010). Each of them, being in the role of a biological indicator has some advantages and disadvantages, which determine the limits of their application when solving the tasks of bioindication because all these groups have a leading role in the overall circulation of substances in the water basin. Organisms, which are usually used as bioindicators are responsible for the self-purification of the water basin, participate in the creation of primary production, transform matter and energy in aquatic ecosystems. Any conclusion based on the results of biological research is based on the whole of all the data obtained, and not on the basis of the individual finding of indicator organisms. During the realization of the research, as well as when evaluating the obtained results, it is

necessary to take into account the possibility of accidental, local pollution in the test site. For example, decomposing plant remains, frog or fish carcasses can cause local changes in the character of the living world of a water basin.

The best developed assessment of the degree of water pollution based on indicator organisms is the saprobity system (tab. 2).

Table 2. Basic characteristics of compatibility zones (Đukić et al., 2013)

Indicator	Polysaprobe zones	Alpha-mesosaprobic
Oxygen conditions	Anaerobic	Semi-anaerobic
Nitrogen compounds	Protein matter	Ammonia, amino acids
Hydrogen-sulphide	A lot	Quite a lot
Rotting	Rotten	Rotten
Bacteria in 1 cm^3 of water	10^9	10^6
Dominance of certain species	Very strong	Little
Diversity of species	Very little	Little
Change of colonies	Catastrophic	Often catastrophic
Oxygen requirements of organisms	Negligible	Weak

Indicator	Beta-mesosaprobic	Oligosaprobic
Oxygen conditions	Aerobic	Aerobic
Nitrogen compounds	Ammonia salts, nitrates, nitrites	Nitrates
Hydrogen-sulphide	Little	None
Rotting	Not rotten	Not rotten
Bacteria in 1 cm^3 of water	10^5	$10 – 10^2$
Dominance of certain species	Significant	Very large
Diversity of species	Significant	Very large
Change of communities	Pretty slow	Slow
Oxygen requirements of organisms	Large	Very large

Among the mentioned groups of indicator organisms higher aquatic plants are the least studied, although they have a number of advantages. They can be seen with the eyes, so they are suitable for research, and also make it possible to visually assess their ecological condition during the hydrobiological examination of water basins. Macrophytes make it possible to determine the trophic properties of water and sometimes the specificity of its chemistry, which is essential for the bioindication of clean waters.

Biological diagnosis of soil

The basis of the principle of soil biological diagnostics is the realization that soil as a living environment forms a unique system with populations of different organisms that inhabit it (Mandić et al., 2019).

Botanical methods of phytoindication and soil diagnostics have been diluted. For example, through the analysis of the composition and structure of plant communities, the distribution of plant indicators or certain indicative properties of certain types of plants, it is possible to determine the type of soil, the degree of its hydromorphism, the development of the process of swamp creation, salt accumulation, etc. Among the plants, indicators of the mechanical and chemical composition of the soil, the degree of enrichment with nutrients, acidity or alkalinity, the depth of thawing of frozen soils or the level of underground water were discovered.

The theoretical assumption of the application of the soil-zoological method for the needs of soil diagnostics is a formulated idea about the "ecological standard" of a species - the need of a species for a certain set of conditions. Each species within its range is found only in those habitats, which ensure the full complex of conditions necessary for the manifestation of life activity (Schloter et al., 2018). The amplitude of variation of certain environmental factors characterizes the ecological harmony of the species. Eurybionts are less suitable for indicator purposes, while stenobionts are good indicators of certain environmental conditions and substrate properties. That setting represents a general theoretical principle in biological diagnostics. However, the use of one species as an indication is not entirely reliable for making correct conclusions (here the "rule of habitat change" and consequently the change of ecological properties of the species has a place). It is better to examine the entire complex of organisms, among which some may be indicators of humidity, others of temperature, others of chemical or mechanical composition. The more common types of land animals are found on the compared plots, the more likely it is to judge the similarity of their regimes, and therefore, the unity of the pedogenetic process. Microscopic forms are less useful - protozoa and microarthropods (ticks, for example). Their representatives are characterized by cosmopolitanism, despite the fact that the soil is not their only habitat: they live in a system of pores, capillaries and cavities, which can be found in any soil. Of the microarthropods, the indicator properties of shell-like (armored) ticks have been best studied. The composition of the complex of their communities depends not only on the soil conditions, but also on the character and floristic

composition of the vegetation, therefore it is promising to use them to indicate harmful effects on the soil.

Communities of large invertebrates (earthworms, centipedes, insect larvae) are particularly valuable and suitable for indication interventions (Menta, Remelli, 2020). So, for example, staphylinids of the genus *Bledius* and black-headed beetles of the genus *Belopus* are typical for salt-salt soils, while some centipedes and lungworms (pulmonary molluscs) are indicators of lime content in the soil. Earthworms *Octolasium lacteum* are indicators of high calcium content in groundwater.

Soil-algological diagnostics is also interesting, based on the thesis that the zonation of soil and vegetation corresponds to the zonation of groups of algae. It is manifested by the overall composition of species and the complex of dominant species of algae, the presence of specific species, the nature of the distribution (spread) along the soil profile and the dominance of certain life forms.

Microbiological and biochemical soil characterization are the most complex parts of soil biodiagnostics. Microorganisms are very sensitive indicators that strongly react to various changes in the environment (Tab. 3). Hence the exceptional dynamism of microbiological indicators. The soil is characterized not only by the composition and abundance of different groups of organisms, but also by their overall activity, as well as the activity of biochemical processes, conditioned by the presence of a certain enzyme, separated during the life activity of plants, animals and microorganisms or accumulated in the soil after the decomposition of cells. Quantitative characteristics of abundance and biomass of different groups of soil organisms, their total productivity, some energy indicators, processes related to the cycling of elements, enzymatic activity of soil. Also the amount and speed of accumulation of some products of life activity of organisms can be indicators of soil biological activity.

Table. 3. Effect of diverse concentrations of lead, copper, cadmium and mercury on the total counts of bacteria (10^6 g^{-1} absolutely dry soil), actinomycetes and fungi (10^5 g^{-1} absolutely dry soil) (Mandić et al., 2010)

Treatments	Concentration (mg dm^{-3})	Total bacterial count	Count of actinomycetes	Fungal count
Control		89.2	75.4	18.2
Pb^{2+}	6.250 0.625 0.125	25.6 ** 65.2 ** 87.8 ns	34.6 ** 35.6 ** 47.6 **	12.0 ** 14.2 * 15.6 ns
Cu^{2+}	6.000 0.600 0.160	27.2 ** 43.6 ** 71.6 *	17.2 ** 43.4 ** 70.6 ns	10.2 ** 16.4 ns 16.6 ns

Cd^{2+}	2.700 0.270 0.027	24.2 ** 27.6 ** 31.6 **	23.6 ** 31.4 ** 45.6 **	6.4 ** 12.6 * 13.0 *
Hg^{2+}	2.220 0.220 0.022	15.8 ** 25.6 ** 30.6 **	13.6 ** 17.4 ** 34.2 **	1.4 ** 6.6 ** 12.2 *

*; **) significant at 0.05 and 0.01, respectively, after the Dunnett test; ns) non-significant

The most basic methods, which allow to evaluate total biological processes based on initial or final products are: methods of determining soil respiration based on O_2 consumption or SO_2 release; determination of nitrogen fixation activity based on acetylene reduction; microcalorimetric measurements to determine the level of thermostability; applied methods with the use of special materials (cellulose, chromatographic paper, cellophane) to assess the speed and degree of decomposition and accumulation of metabolic products, for example amino acids. A special group consists of methods for determining the potential activity of certain enzymes in soils (actually activities, not quantities).

Conclusion

Various bioindicative organisms can be used for rapid assessment of environmental quality. For the bioindicative assessment of air quality lichens are most often used; water - primarily planktonic microorganisms, as well as benthic organisms, including macrophytes; and soils - microbiological, enzymatic, lichenological, zoological and phytoindicative methods.

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CONTRIBUTION TO THE KNOWLEDGE OF THE ANTIOXIDANT POWER, PHENOLIC AND MINERAL COMPOSITION OF *SANGUISORBA MINOR* SCOP.

Gorica Djelic[1], Milica Pavlovic[2], Snezana Brankovic[3], Dusko Brkovic[4], Zoran Simic[5], Vesna Velickovic[6]

Abstract: In this study, extracts of the aerial partsof *Sanguisorba minor*Scop. wereanalysed for antioxidant activity, quantification of the total phenolic and flavonoid and bioaccumulation potential of heavy metals.The total phenols amounts was the highest at acetone´sextracts of root (117.27 mg GAE /g dry weight), stem and leaf (133.61 mg GAE /g dry weight) and flower (116.77 mg GAE /g dry weight) and total flavonoids acetone´sextractsof flower (50.69 mg RU/g). The highest DPPH-scavenging capacity had flower methanol extract 7.08 (IC_{50} μg/ml). The plant accumulates large amounts of Zn, Ni and Cu in the root, stem and leaf and flower. Otherwise Mn, Cr and Pb are represented in whole plant.

Key words:*Sanguisorba minor*, antioxidant potential, total phenolics, metals

Introduction

Medicinal plants have a long tradition in all nations, plant-based treatment has been used in natural medicine since ancient times, and experiences and knowledge are passed down from generation to generation. What was previously used as a form of alternative treatment, today becomes a supplement to conventional treatment methods, as a result of this trend there is a great interest in medicinal and aromatic plants.

In this paper, the medicinal properties will be examined of the plant species *Sanguisorba minor*Scop. specie belong to the *Rosaceae*family. Plant species of this family exert a wide range of biological activities such as antioxidant (Barral-Martinez*et al*. 2021, Šola *et al*. 2022).

Sanguisorba minorScop.,garden burnet(Grlić, 1990), small burnet (Mišić, Lakušić, 1990.), is an edible perennial herbaceous plant. Specie belonging to

[1,2,3,5]UniversityofKragujevac, FacultyofScience Kragujevac, Radoja Domanovića12, Kragujevac, Serbia
[4]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia
[6] University of Kragujevac, Faculty of Technical sciences, Svetog Save 65, Čačak, Serbia (vesna.velickovic@ftn.kg.ac.rs)

the *Rosaceae* family.It has composite leaves with dentate leafleat, one of them, terminal. It is widespread in central and southern Europe, in southwest Asia and northwest Africa(Mišić, Lakušić, 1990.),.

Sanguisorba minor, plant specie has not been sufficiently studied in terms of its antioxidant effect. Certain investigations of *Sanguisorba* species indicate they are rich sources of bioactive compounds and shows significant beneficial effects (Grbz, 2005.Zhao*et al.* 2017, Ayoub, 2003).Scientific studies indicate that *S. minor* has numerous positive effects that are related to the content of bioactive compounds (Ayoub, 2003, Arıhana*et al.* 2015).

The present study aimed to examine the phenolic composition and potential antioxidative properties of *S. minor*. The plant sample was taken from a location is polluted. Considering the greater pollution of the environment and the presence of various metals and other toxic side products of various branches of industry and traffic in the soil, due to the ability of plants to adopt these ingredients, it is necessary to determine their content so that the plants, in addition to being useful, do not have a negative effect. For each element, the maximum limit concentration that a plant can contain without having a negative effect is prescribed and defined (WHO, 1994). In this study, we aimed to determine the bioaccumulation potential of ten heavy metals.

Matherials and methods

Plant material from Sanguisorba minor was collected from the tailings in Kosovska Mitrovica. The plant material, consisting of both underground and surface parts of the plant in the flowering phase, was air-dried and prepared for analysis. From each sample of dried plant material S. minor, part of material was finely grinded, then used for analysis.

Determination of total phenol content of the extracts

The total phenolic content of the extract was determined by the Folin–Ciocalteu method as described by Peter *et al.*, 2011. The test exemplars were prepared in triplicate for each analysis. The absorbance was determined using spectrophotometer, λ max = 725 nm. The level of phenolics was expressed in terms of gallic acid equivalent per gram of plant extract (mg GA·g^{-1}).

Determination of total flavonoid content of the extracts

The concentration of flavonoids in plant extracts was determined by the Quettier-Deleu*et al*. 2000. The absorbance was determined using spectrophotometer at λ max = 430 nm. Rutin (mg/ml) was used to prepare the standard curve, the level of flavonoid was expressed in terms of rutin equivalent per gram of plant extract (mg RU·g^{-1}).

Determination of the antioxidant activity of the extracts

The free radical scavenging activity of the three extracts (methanol, acetone and ethyl acetate) of *Sanguisorbaminor*root and herba was analyzed using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) as described by the Takao *et al*., 1994. The absorbance was measured at λ max =517 nm. Antioxidant activity was expressed as the 50% inhibitory concentration (IC_{50} values at μg/mL).

Analysis of heavy metals in samples of plant

The concentration and content of heavy metals in the aerial parts and roots were determined by atomic absorption spectrophotometry. Heavy metals, including manganese (Mn), iron (Fe), lead (Pb), nickel (Ni), chromium (Cr), copper (Cu), cadmium (Cd), zinc (Zn), calcium (Ca), and magnesium (Mg), were measured in both underground and surface parts of the plant. The analysis of heavy metals was conducted at the Laboratory for Analytical Chemistry at the Faculty of Science, University of Kragujevac. Five samples were prepared for each analysis, and the atomic spectrophotometer (Perkin Elmer 3300) was used. The mean, standard deviation, bioaccumulation factor (BCF), and translocation factor (TF) were determined according to Kabata-Pendias (2001).

Data analysis

Statistical analyses of data are obtained by using, SPSS for Windows, version 21. The values of the correlation coefficient (r) were determined using the Pearson correlation coefficient. Statistically significant difference was defined as $p < 0.05$.

Results and discussion

Determination of total phenolic, total flavonoid contentand antioxidant activity assay

Total phenolic (TPC), flavonoid content (TFC) and antioxidant capacity of extracts, evaluated by three different solvent as well as are presented in Table 1. The values obtained for the concentration of total phenolic are presented in first part of Table 1. The results of the total phenol content in the tested parts of plant are expressed as gallic acid equivalent (the standard curve equation:y=76.735x+0.069, r^2 = 0.994); the values are expressed as mg GAE/g of extract. Results of total amount of phenols in root, stem and leaf and flower as the average value and standard deviation calculated based on measurments in triplicate. The highest concentration of phenols in root were extracted with acetone (117.27±1.41 mg GAE/g). The highest concentration of phenols in stem and leaf were extracted with acetone (133.61±0.85 mg GAE/g). The highest concentration of phenols in flower was extracted with methanol (152.33±1.01 mg GAE/g).Differences between this two solvents were significant in terms of phenols content in different parts of the herb ($p<0.05$). Our study shows a higher phenol content in extracts extracted with acetone. In other studies, the extracts were extracted with acetone as solvent with the significant amounts of total phenolics(Do *et al*. 2014, Aline*et al.* 2014).

Table 1. Total phenolic (TPC) and flavonoid content (TFC) and antioxidant capacity of *Sanguisorba minor*

Part of plant	Solvent	TPC (mg GAE g^{-1})	TFC, (mg RU g^{-1})	IC_{50} (µg/mL)
Root	Methanol	81.72±1.73	14.42±0.42	46.12±1.14
	Acetone	117.27±1.41	13.09±1.13	40.9±0.5
	Ethyl-acetate	64.44±0.85	11.02±0.61	88.72±1.03
Stem and leaf	Methanol	120.5±1.92	15.66±0.54	47.27±0.01
	Acetone	133.61±0.85	22.38±0.54	9.4±±0.01
	Ethyl-acetate	115.22±3.34	8.78±0.07	19.1±0.53
Flower	Methanol	152.33±1.01	33.57±1.85	7.08±0.15
	Acetone	116.77±1.71	50.69±1.64	21.33±0.57
	Ethyl-acetate	95.88±1.57	32.92±1.25	10.45±1.05

The results of the total flavonoids in the tested parts of plant are expressed as rutin equivalent, (the standard curve equation:y=14.78x+0.027, r^2 = 0.995); the

values are expressed as mg RU/g of extract.Measurmentswerenin triplicate. The highest concentration of flavonoids in root were extracted with methanol (14.42±0.42 mg RU/g9.The highest concentration of flavonoids in stem and leaf were extracted with non-polar acetone (22.38±0.54 mg RU/g).The highest concentration of flavonoids in flower were extracted with acetone (50.69±1.64 mg RU/g).Previous studies reported that different solvents significantly affected flavonoids especially acetone(Dailey and Vuong, 2015, Do Q.D.,2014).

The antioxidant activity is expressed in terms of IC_{50} (μg/ml) values (Table 1). The obtained values of antioxidant activity examined by DPPH radical scavenging activity range from 7.08 ± 0.15 to 21.33 ± 0.57 μg/ml. The largest capacity to neutralized DPPH radicals was measured in ethyl-acetate extract from root (88.72 ± 1.03 mg/ml), methanol extract from stem and leaf (47.27 ± 0.01 mg/ml) and acetone extract from flower (21.33 ± 0.57 mg/ml). Our results compared to (Pereira *al*.,2011) whose IC_{50} value (30 ± 0.0 mg/ml). This result suggest that the flowers of *Sanguisorba minor* are likely to have high antioxidant capacity, more than root, stem and leaf.

Based on the mean values of the metal concentration in the soil, we can compare them in the following order: Fe> Mg>Ca>Mn>Pb> Cr> Ni> Zn> Cu. The values of the concentration of heavy metals were much higher in comparison with Table 3, except for Cu (113.88 mg kg^{-1}). Based on the concentrations (Soriano *et al*., 2010) the tested soil is moderately polluted. The study (Hasanović*et al.,* 2022) coincide wi*ث*th our results. In that study *Sanguisorba minor*grows on soil with high concentrations of heavy metals. Our results (Table 2) showed that in the soil on which the tested specie grew had a higher value than the maximum allowed concentration of metals we tested and a high content of Fe (7807mg kg^{-1}). If iron is accumulates in plants to high levels than is toxic, it can act catalytically via the Fenton reaction to generate hydroxyl radicals, which can damage lipids, proteins and DNA (Connolly, Guerinot, 2002).

Table 2. The content of investigated metals (mg kg^{-1}) in soil and specie *Sanguisorba minor*

Metal	**Soil (mg/kg)**	**Root (mg/kg)**	**Stem and leaf herb (mg/kg)**	**Flower (mg/kg)**
Mn	2685.58±16.06	458.78±0.94	162.58±0.81	475.08±27.51
Ni	199±0.97	21.26±0.32	1.25±0.02	26.2±0.37
Ca	3649.76±31.20	21277±55.20	17253.8±83.59	27694.38±66.29

Mg	4540.54±23.4	8829.64±78.07	7209.8±68.34	11089.12±28.21
Fe	77363.08±682.37	7807.36±83.25	723.32±1.72	7276.08±38.16
Zn	176.24±0.78	92.74±0.53	36.84±0.49	77.76±0.74
Cr	453.36±1.36	72.32±0.81	5.84±0.05	70.32±0.51
Pb	873.66±2.057	121.74±0.77	22.32±0.43	87.86±0.75
Cu	113.88±0.8	19.32±0.39	7.47±0.05	18.38±0.42

Table 3. Classification of contaminated soil according to heavy metal content (mg kg^{-1}) (Soriano *et al.*, 2010; Kabata – Pendias, 2011)

Chemical element	**MPK – maximal permitted concentrations mg/kg**
Manganese (Mn)	2 000
Iron (Fe)	50 000
Lead (Pb)	100
Nickel (Ni)	50
Chromium (Cr)	100
Copper (Cu)	100
Cadmium (Cd)	3
Zinc (Zn)	300
Calcium (Ca)	-
Magnesium (Mg)	-

Table 4. Bioconcentration factor (BCF) and Translocation factor (TF) of specie *Sanguisorba minor*

Metal	**Bioconcentration**	**Translocation root/herba**	**Translocation root/flower**
Mn	0.170830882	0.354374646	1.035529012
Ni	0.107373737	0.058889934	1.232361242
Ca	5.829753189	0.81090557	1.301598895
Mg	1.944623327	0.816545182	1.255897183
Fe	0.100918423	0.092645914	0.931951389
Zn	0.526214253	0.397239595	0.838473151
Cr	0.159520028	0.080807522	0.972068584
Pb	0.139344825	0.183341548	0.721701988
Cu	0.169652266	0.386956522	0.951345756

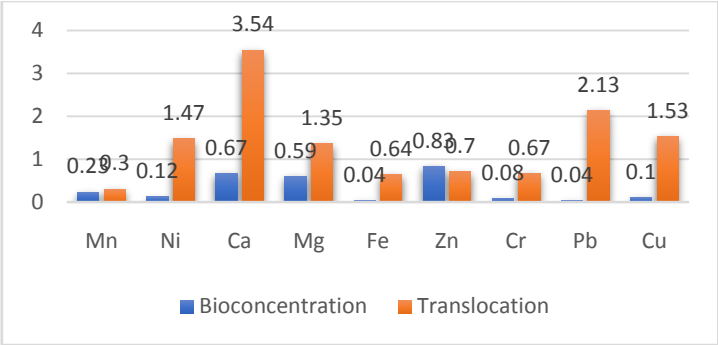

Figure 1.Bioconcentration factor (BCF) and Translocation factor (TF) of specie*Sanguisorba minor*

		root									herba								
		Mn	Ni	Ca	Mg	Fe	Zn	Cr	Pb	Cu	Mn	Ni	Ca	Mg	Fe	Zn	Cr	Pb	Cu
root	Mn	1																	
	Ni	-0.5	1																
	Ca	**0.92***	-0.46	1															
	Mg	-0.02	-0.41	-0.32	1														
	Fe	0.07	0.68	-0.07	-0.13	1													
	Zn	0.36	0.49	0.22	-0.01	0.83	1												
	Cr	**-0.89***	0.77	-0.82	-.008	0.5	0.06	1											
	Pb	0.17	-0.39	0.03	0.72	0.29	0.24	-0.08	1										
	Cu	-0.35	0.04	-0.07	-0.22	0.33	-0.16	0.41	0.32	1									
herba	Mn	0.8	0.1	0.7	-0.38	0.34	0.7	-0.5	-0.1	-0.36	1								
	Ni	-0.21	-0.52	-0.24	0.23	**-0.95***	**-0.88***	-0.23	-0.28	-0.28	-0.56	1							
	Ca	-0.43	0.19	-0.31	0.13	0.59	0.12	0.59	0.59	0.87	-0.39	-0.46	1						
	Mg	-0.14	0.86	0.1	-0.75	0.65	0.53	0.46	-0.51	0.1	0.45	-0.64	0.07	1					
	Fe	0.56	-0.69	0.76	-0.36	-0.56	-0.45	-0.73	-0.15	0.13	0.22	0.31	-0.29	-0.27	1				
	Zn	0.58	-0.37	0.81	-0.66	-0.36	-0.23	-0.64	-0.41	0.04	0.47	0.1	-0.4	0.1	**0.9***	1			
	Cr	**0.88***	-0.11	0.86	-0.23	0.36	0.67	-0.6	0.17	-0.14	**0.93***	-0.62	-0.17	0.25	0.34	0.48	1		
	Pb	0.5	0.01	0.75	-0.68	0.36	0.31	-0.26	-0.04	0.44	0.62	-0.59	0.17	0.47	0.55	0.71	0.72	1	
	Cu	0.49	-0.48	0.44	-0.11	-0.78	-0.36	-0.74	-0.5	-0.7	0.29	0.61	**-0.93***	-0.29	0.57	0.58	0.15	-0.09	1

Figure 2. Pearson's Correlation of heavy metals among root and herbaof specie*Sanguisorba minor*

According to our results, Mn(458.78mg kg^{-1}) and Zn (92.764 mg kg^{-1}) in root is in much lower concentration(Soriano *et al*., 2010; Kabata-Pendias, 2011). Results obtained for Pb (121.74 mg kg^{-1} in root, and 22.32 mg kg^{-1}stem and leaf and 87.86 in flower) is lower that the maximum allowed concentration in plant except the content in the root where is higher contrecationtham allowed (Soriano *et al*., 2010; Kabata - Pendias, 2011). The obtained results for heavy metals show that BAC<1, which means that *S. minor*heavy metal excluders. The BF and TF of Zn were both less than 1 (0.62, 0.96), which coincides with the research (Kothe and Varma, 2012) for *S. minor* BF and TF of Zn were both less than 1 (0.7, 0.7). This indicate that *S. minor*roots are able to take up the metals (in dissolved form from low levels in the soil).

Conclusion

The impact of the chemical composition and bioactivities of different parts of the Sanguisorba minor plant was assessed in order to highlight its important role in human diet and health. Based on the obtained results, the investigated soil was found to be moderately polluted with metals such as Mn, Pb, Ni, Cr, Fe, Cd, Cu, Ca, and Mg. However, the Sanguisorba minor plant was found to grow successfully on this type of soil. The solvents used in this study revealed that acetone was a very efficient medium for extraction, as it resulted in the highest amounts of total phenols, flavonoids, and antioxidant activity. All these results indicate that Sanguisorba minor could be an important source of bioactive compounds, with good antioxidant properties, and thus can be used in various treatments. However, it is necessary to carry out detailed investigations beforehand, especially if the plant species originates from an area contaminated with metals.

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CELERY (*APIUM GRAVEOLENS* L.) AS A SOURCE OF PHYTOCHEMICALS WITH ANTIOXIDANT AND ANTIBACTERIAL EFFECTS

Vesna Đurović[1], Leka Mandić[1], Marija Igrošanac[1], Mirjana Radovanović[1], Marijana Pešaković[2], Jelena Mladenović[1], Dragutin Đukić[1]

Abstract: The aim of this work was to investigate the content of phytochemicals in celery root and its antioxidant and antibacterial potential. The content of total phenols in celery root was 2.99 mg GAE g^{-1}, total flavonoids 1.38 mg RE g^{-1}, total tannins 1.87 mg GAE g^{-1} and vitamin C 12.7 mg 100 g^{-1}. Antioxidant activity measured by the DPPH method and expressed in % inhibition were 42.26 % or 2.38 μmol TE g^{-1}. Antibacterial activity was tested on pure cultures of *Enterococcus faecium, Listeria monocytogenes, Staphylococcus aureus, Salmonella enteritidis, E. Coli* and *Serratia marcescens*. *Listeria monocytogenes* showed the highest sensitivity while the *Salmonella enteritidis* and *E. coli* were the most resistant.

Keywords: celery, antioxidant, antibacterial activity

Introduction

Many aromatic plants have been used as vegetable and flavoring ingredients in cooking and in traditional medicine. Celery (*Apium graveolens* L.) is an aromatic plant from the Apiaceae (Umbelliferae) family. The whole plants including leaf, stem, seed and root are used in cooking as soups and salads due to its aroma and essential oil (Aboody, 2021). According to the assessment report of the Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency on *Apium graveolens* has been known as a medicine since ancient times in Greece.

Celery is a good source of vitamins A and C, carotenes, tocopherols and secondary metabolites such as flavonoids (including quercetin, apigenin, kaempferol, isorhamnetin and luteolin), alkaloids, terpenoids, tannins, saponin, phenolic acids (caffeic acid, p-coumaric acid, ferulic acid) and volatile compounds (limonene, myrcene, α-pinene, β-selinene) (Kooti et al., 2014; Al-Asmari et al., 2017). Thanks to the presence of these components, celery has a high antioxidant potential

[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia (djurovicvesna@yahoo.com)
[2]Fruit Research Institute Čačak, Kralja Petra I 9, 32102 Čačak, Serbia

(Kooti and Daraei, 2017) and the ability to remove OH and DPPH radicals (2,2-diphenyl-1-picrylhydrazyl) (Kooti and Daraei, 2017; Emad et al., 2022).

Celery is used to treat cardiovascular diseases, strengthen the heart and lower blood pressure. It has anticoagulant properties. Celery root was used to reduce potassium and increase calcium in cardiac tissues (Khalil et al., 2018). Celery has many pharmacological activities: antimicrobial, antifungal, antiparasitic, anti-inflammatory, antioxidant, antidiabetic, anti-spasmolytic, hepatoprotective, cardioprotective, neuroprotective, cytoprotective and analgesic activity, etc. (Khairullah et al., 2021). The essential oils of celery have anti-bacterial effects on *Streptococcus faecalis, Staphylococcus albus, Staphylococcus aureus, Salmonella typhi, Shigella dysenteriae* and *Streptococcus pyogenes* (Khalil et al., 2018).

The content and chemical composition of celery depend on genetic and environmental factors, as well as applied agrotechnical measures such as fertilization, irrigation, growing method and harvesting method (Powanda et al., 2015).

In this study, celery root was investigated as an aromatic herb in traditional Serbian cuisine, which is known to have strong pharmacological activity and a high content of antioxidants. They were tested for their content of biologically active compounds (the content of ascorbic acid, tannin, phenolic and flavonoid compounds) and their antioxidant and antibacterial activity.

Material and methods

Sample preparation and analysis

The samples (*Apium graveolens* L.) were harvested at full maturity in 2021 from individual producers, who cultivated them in the vicinity of the town Trstenik, Rasina district, Serbia. The sample (roots) was ground and dried. Samples were extracted with 50% ethanol at 25 ℃in an ultrasonic bath for 30 min., and after that 24 h maceration at room temperature. The extracts were filtered and then centrifuged at 5000rpm for 10 min. The clear supernatants were used for the determination of the total phenolic, flavonoids, tannin content and antioxidant activity. For antibacterial activity, the extracts were evaporated in a nitrogen atmosphere under a vacuum, in a water bath at 60 ˚C.

Determination of dry matter content in the samples was performed by gravimetric method at 105˚C , drying to constant mass. The ash content was determined by burning at 550 ˚C for 14 h.

The total phenolic content was determined using a modified Folin-Ciocalteu colorimetric method (Singleton et al., 1999; Liu et al., 2002). The tannin content in the sample was determined using (PVPP) insoluble polyvinyl-polypirrolidone (Makkar, 2003). The flavonoid content (FC) was determined using Zhishen et al. (1999) method. The antioxidant capacity of the extracts was studied through the evaluation of the free radical-scavenging effect on the 1.1-diphenyl-2-picrylhydrazyl (DPPH) radical (Brand-Williams et al., 1995). The estimation of ascorbic acid was determined by the titration method (Tillmans' method).

The antibacterial properties of plant extract were tested against Gram-positive bacteria (*Enterococcus faecium* ATCC 6057, *Listeria monocytogenes* ATCC 13932 and *Staphylococcus aureus* ATCC 25923) and Gram-negative bacteria (*Salmonella enteritidis* ATCC 13076, *E. coli* WDCM 0013 and *Serratia marcescens* ATCC 43862). The strains used in the tests were sown 24 h before the experiments. After this period, the bacterial inoculum was suspended in saline, corresponding to 0.5 of the McFarland scale, approximately 1.5×10^8 CFU/mL. The minimum inhibitory concentration tests were performed using the microdilution method with resazurin as an indicator (Javadpour et al., 1996). Resazurin is a molecule that serves as a redox indicator. In response to the metabolic activity of living cells, resazurin (blue) is reduced to resorufin, which has a pink color and is fluorescent. The MIC is defined as the lowest concentration at which no growth is observed (Oliveira et al., 2022).

Results and discussion

The content of moisture, dry matter and ash in celery roots was shown in Table 1. It can be especially noted that the celery root has a high content of ash or mineral substances (8.94%). This is in accordance with literature data showing that celery root has a significant content of minerals such as K, P, CA, Se, Ni and others (Qureshi et al., 2014). Researchers state that every part of the plant contains minerals necessary for the human body, with the most of them being in the leaves (Dong and Zhao, 2004).

Table 1. Content of moisture, dry matter and ash in the samples

Sample	Moisture content, %	Dry matter content, %	Ash content, %
Celery root	7.22	92.78	8.94

In this study total phenols, flavonoids, tannins and vitamin C content as well as antioxidant activity were detected in celery roots. The results are shown in Table 2.

Table 2. Content of total phenols, flavonoids, tannins, vitamin C and antioxidant activity

Samples	Total phenolics, mg GAE g^{-1}	Total flavonoids, mg RE g^{-1}	Total tannins, mg GAE g^{-1}	DPPH, % inhibition	DPPH, µmol TE g^{-1}	Vitamin C, (mg/100g)
Celery root	2.99±0.07	1.38±0.17	1.87±0.01	42.26±3.61	2.38±0.24	12.70±0.15

The vitamin C content of celery root was 12.7 mg $100g^{-1}$. Similar results for vitamin C are reported by Qureshi et al. (2015). However, the content of vitamin C is significantly higher in the leaves compared to the roots (Meng-Yao et al., 2017). The content of total phenols in celery root was 2.99 mg GAE g^{-1}, total flavonoids 1.38 mg RE g^{-1} and total tannins 1.87 mg GAE g^{-1} (Table 2). Tang et al. (2015) and Jung (2011) reported amounts of phenolic compounds in celery (4.64 g GAE 100 g^{-1}). Antioxidant activity measured by the DPPH method and expressed in % inhibition were 42.26% or 2.38 µmol TE g^{-1} (Table 2). Phenolic compounds and flavonoids are mainly responsible for the antioxidant activity of plants (Masuoka et al., 2012). There are many studies that have demonstrated the antioxidant activity of Apium plants (seeds, roots and leaves) in vitro and in vivo (Salehi et al., 2019).

This study also investigates the antimicrobial activity of *Apium graveolens* against six bacterial strains. The MIC and MBC values of celery extract were used to evaluate the bacteriostatic and bactericidal properties. MIC and MBC values were determined based on bacterial growth on MHA (Muller Hinton Agar). MIC and MBC of celery root extract are shown in Table 3.

Table 3: The effect of the extract on the tested bacterial cultures

Bacteria	MIC mg/mL	MBC mg/mL	Dovicin µg/mL
1. *Enterococcus faecium* ATCC 6057	100	>100	<0,24
2. *Listeria monocytogenes* ATCC 13932	50	>100	<0,24
3. *Staphylococcus aureus* ATCC 25923	100	>100	0,24
4. *Salmonella enteritidis* ATCC 13076	>200	>200	1,95
5. *E.coli* WDCM 0013	>200	>200	0,92
6. *Serratia marcescens* ATCC 43862	100	>100	<0,24

The results showed different degrees of inhibition of antibacterial activity against some of the tested microorganisms. From the obtained results it can be seen that *Listeria monocytogenes* ATCC 13932 showed the highest sensitivity while the most resistant were *Salmonella enteritidis* ATCC 13076 and *E. coli* WDCM 0013.

The structure of the cell wall of Gr^- bacteria is more complex compared to the structure of Gr^+ bacteria, because it contains a thin layer of peptidoglycan, surrounded by an outer membrane made of lipopolysaccharides, while in Gr^+ bacteria there is no outer membrane, but a thick layer of peptidoglycan (Silhavy et al., 2010). The strong hydrophobic outer membrane of Gr^- bacteria acts as a strong permeability barrier (Nikaido, 2003) and limits the diffusion of active substances from the agent (Pierozan et al., 2009).

A. graveolens contain tannins, phenols, flavonoids, vitamin C (Table 2), in accordance with that, literature data show that phytochemicals in plants have antimicrobial activity, especially flavonoids (Mickymaray, 2019). The main mechanism of the antibacterial action of the phenols and flavonoids is DNA inhibition, membrane degradation and deterioration of energy metabolism (Al Aboody et al., 2020). This is consistent with literature data which show that extract of celery leaves and roots demonstrated antibacterial activity against different bacteria (Sipailiene et al., 2005, Emad et al., 2022) and that Apium plants have a role in the prevention of microbial growth (Maxia at al., 2012; Edziri at al., 2012; Baananou et al., 2013).

Conclusion

Recently, there has been a growing interest in using natural antioxidants from plant sources instead of synthetic compounds. Various plants from the Apiaceae family including *Apium graveolens* present a good source of antioxidants such as phenolic acids, flavonoids, tannins, stilbenes, coumarins, lignans, carotenoids, tocopherols and ascorbates. Of these phenolic compounds, especially phenolic acid and flavonoids make the main contribution to scavenging free radicals as well as inhibition of different gram-positive and gram-negative bacteria.

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KINETIC AND EQUILIBRIUM STUDIES OF BIOSORPTION OF Cd(II) IONS USING SILICA-ALGINATE-YEAST COMPOSITE

Slobodanka Stanojević-Nikolić[1,2], Milan P. Nikolić[1], Marina Šćiban[2], Vladimir V. Srdić[2], Vladimir B. Pavlović[3]

Abstract: Viable *Saccharomyces cerevisiae* cells were immobilized by silica-alginate matrix and obtained spherical composite particles and used for biosorption of Cd(II) ions. The obtained composite displayed high cadmium removal efficiency of 99.2, 95.7, 88.3 and 78% in the successive four adsorption steps. The adsorption capacity after four step of Cd(II) removal was ~14,2 mg of Cd(II) per g of biosorbent. Pseudo-second-order kinetic agree well with the experimental values suggesting both adsorption and ionic exchange are simultaneously performed on the surface of used biosorbent.

Keywords: biosorption, heavy, *Saccharomyces cerevisiae,* metals, biocomposite

Introduction

Global industralization and urbanization are responsible for increasing amounts of toxic pollutants in the effluents and soil. Environmentally relevant most hazardous substances which contaminate environment, expecially water, are heavy metals, due to their persistent, non-biodegradable, and toxic nature (Hazrat et al., 2019). Cadmium is highly toxic, non-essential heavy metal which may couse serious health problems to all living organisms (Genchi et al., 2020). Due to its ability to be accumulated in living organisms, it has harmful impact on lungs, kidneys, liver, reproductive organs etc. Both short-term or long-term exposure to cadmium can cause serious health problems such as renal and hepatic dysfunction, pulmonary edema, testicular damage. Thus, it is very important to prevent cadmium from getting into the environment from industrial effluents. It is well known that both geogenic and anthropogenic

[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia (slobodankasnikolic@gmail.com)

[2]University of Novi Sad, Faculty of Technology Novi Sad, Blvd. Cara Lazara 1, 21000 Novi Sad, Serbia

[3]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia.

sources can elevate Cd concentrations in soils and groundwater. Significant sources of natural Cd emissions are hydrothermal vents, weathering of rocks and airborne soil particles from deserts, sea spray, forest fires, biogenic material and volcanoes. Sources of anthropogenic Cd emissions are non-ferrous metal production, fossil fuel combustion, phosphate fertilizer manufacturing, iron, steel, and cement production and road dust. Municipal and sewage sludge incineration can also release cadmium into air, water, and soil (Kubier et al., 2019). Furthermore, Cd can be found in products such as pigments, coatings, platings, stabilizers for polyvinyl chloride (PVC) and alloys. Phosphate fertilizers contain between 36 and 77 mg Cd per kg P_2O_5, making them one of the most important sources of Cd contamination in soils (Kubier et al., 2019).

The application of biosorption for removal of heavy metals from wastewater has been concidered as an effective procedure for water treatment (He and Chen, 2014). Major advantages of biosorption over conventional treatment methods (precipitation, adsorption, reduction, coagulation, and membrane filtration) is low cost, eco-friendliness, high binding ability, high efficiency of removal metal ions in low concentration, low biological sludge, etc (Aryal 2019). Low cost and eco-friendly biosorption of heavy metal ions using nonpathogenic microbial biomass is generally regarded as safe and it is receiving more attention in recent years (Mishra et al., 2020).

The immobilization of microbial cells on numerous matrices via entrapment was found to enhance bioremediation of heavy metals (Keskin et al., 2018) and dyes (San et al., 2014) from wastewater. Immobilized microbial cells have following advantages over the free cells in metals biosorption process: easy manipulation, higher stability of composites, easy separation after biosorption procedures, potential use in real continuous systems (Giese 2020).

In order to make advanced biosorbents for removal of cadmium ions from aqueous solutions, we have developed processing method for immobilization of non-pathogenic and safe *Saccharomyces cerevisiae* cells in silica-alginate composite material. Kinetic and equilibrium studies of biosorption of Cd^{2+} ions using silica-alginate-yeast composite have been investigated.

Materials and methods

0.5 g of the yeast cell biomass was mixed with 2 ml of silicate solution (having a Na_2O/SiO_2 molar ratio of 0.4 and SiO_2 concentration 362 g/L) and 8 ml of 5% w/v alginate solution. The mixture of sodium silicate, alginate and microbial cell was slowly added (Figure 1b) into previously prepared 0.1 M

calcium chloride solution (Figure 1). The silica-alginate- *S. cerevisiae* (SA-Sc) composite particles were washed and dried at room temperature.

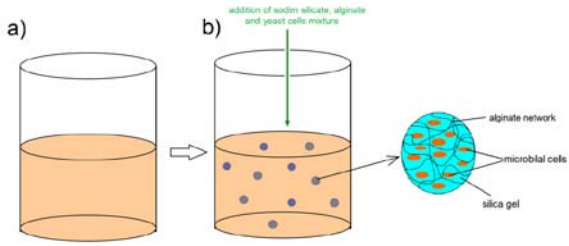

Figure 1. Preparation of SA-Sc composite particles (Stanojević-Nikolić et al., 2022)

The kinetics of adsorption of cadmium ions from aqueous solution by alginate-silica-microbial cell composite were investigated by dispersing of 0.2 g of this composite material in 10 mL of 1 mmol/L aqueous cadmium(II) sulphate solution at a constant temperature (25°C) and pH=6. After appropriate contact time between 5 to 120 minutes, the small aliquots of sample supernatant were diluted in 1% (w/w) nitric acid.

The cadmium concentration, both in the initial and final solution, was determined by flame atomic absorption spectrometry using Perkin Elmer AAnalyst 200 system after calibration with stock solutions in the range of concentration of 0.5-4 mg/L. The equilibrium amount (adsorption capacity) (q_e) of cadmium ions adsorbed on sorbent phase (mg Cd^{2+}/g dry sorbent) was calculated in the following way:

$$q_e = \frac{(C_0 - C_e)}{m} \cdot V \tag{1}$$

where C_0 (mg/L) is the initial concentration of Cd^{2+}, C_t (mg/L) is the concentration of Cd^{2+} at time t, V (L) is the total volume of solution and m (g) is the mass of bisorbent. All experiments were performed in triplicates and the mean values were used in the data analysis.

In order to investigate the mechanism of biosorption, characteristic constants of adsorption rate were determined at individual concentrations for silica-alginate-yeast composite and at different time by using a pseudo-first-order equation (Eq. (2)) of Lagergren based on solid capacity, and pseudo-second-order equation (Eq. (3)) based on solid phase adsorption:

$$ln(q_e - q_t) = -k_t + lnq_e \tag{2}$$

$$\frac{t}{q_t} = \left(\frac{1}{q_e}\right)t + \frac{1}{k_2 q_e^2} \quad (3)$$

where q_t and q_e (mg/g) are the amounts of the metal ions biosorbed at time t (min) and equilibrium (mg/g), respectively and k_1 is the rate constant (min^{-1}) of the pseudo-first model equation, k_2 (g/mg min) is the rate constant of the second order equation. The biosorption rate constants (k_1) can be determined experimentally by plotting of $\ln(q_e - q_t)$ versus t. On the other hand, the biosorption rate constants (k_2) can be determined experimentally by plotting t/q_t versus t.

The size and morphology of the particles were examined using a scanning electron microscope (SEM, JSM-6390 LV JEOL, operating at 30 kV) coupled with energy dispersive spectrometer (EDS) (Oxford Instruments X-MaxN). Prior to SEM imaging, the samples were sputtered with gold.

Results and discussion

Figure 2 shows SEM micrographs of silica-alginate-*S. cerevisiae* (SA-Sc) composite particles. Average particle size is about 3 mm (Fig. 2a). The microbial cells are clearly visible on the surface of particles (Fig. 2b). EDS insertion on Fig. 2b shows the presence of high levels of cadmium on gthe surface of biosorbent.

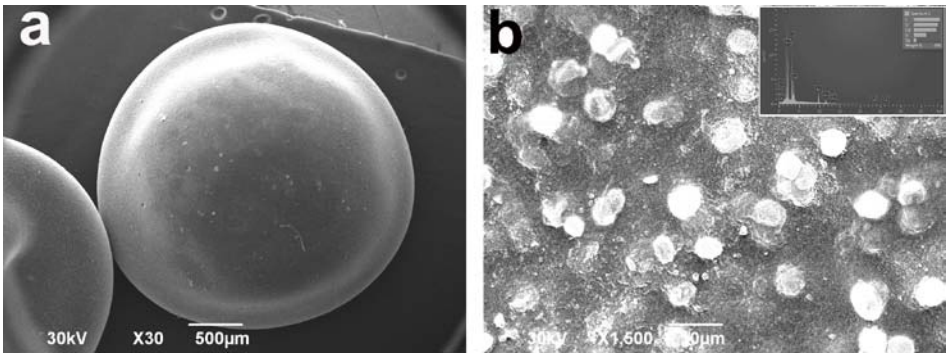

Figure 2. SEM micrograph of SA-Sc composite

The effect of reaction time on cadmium binding capacity of SA-Sc composite particles is shown in the Figure 3. The saturation capacities in four cycles was reached after approximately 20, 50, 70 and 90 minutes and have values of 3.9, 3.8, 3.6 and 2.9 mg/g, respectively.

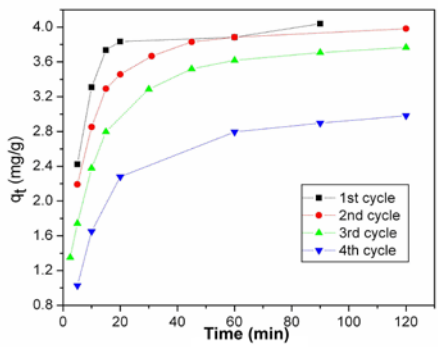

Figure 3. Effect of reaction time on cadmium binding capacity of SA-Sc composite in four adsorption cycles

The removal efficiency of Cd(II) ions in successive four steps using silica-alginate-yeast composites is shown on Table 1. It shows high removal efficiencies which gradually decreased from 99.22% for the first cycle to 78% for the fourth cycle.

Table 1. Removal efficiency of cadmium ions in four adsorption cycles

The cycle number	1st cycle	2nd cycle	3rd cycle	4th cycle
Removal efficiency (%)	99.22	95.77	88.28	78.00

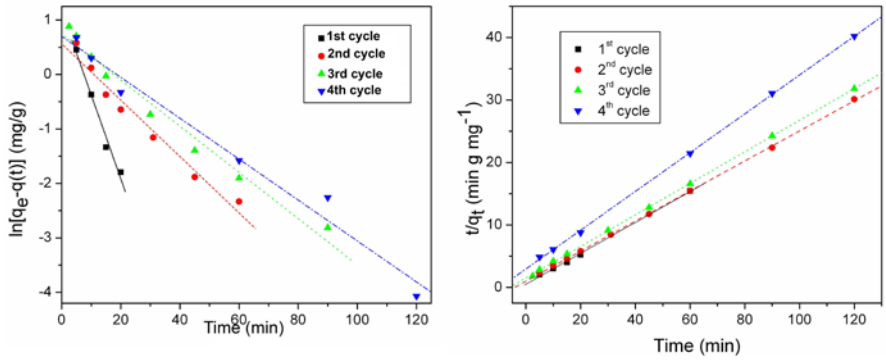

Figure 4. Application of two models to the adsorption kinetics of Cd(II) ions onto SA-Sc composite: pseudo-first order model (left); pseudo-second-order model (right)

The graphics application of the pseudo-first-order and pseudo-second-order kinetics on the obtained experimental results is shown on Figure 4. The corresponding pseudo-first-order and pseudo-second-order rate constants are

calculated from graphics shown in Figure 4 and listed in Table 2. Table 2 shows that these constants decreased as the cycle number increased. The calculated values of q_e for pseudo-second-order kinetic agree well with the experimental values, with values of $R^2 > 0.999$ indicating that this model could be applied over the entire adsorption process. Pseudo second-order kinetic suggests that both adsorption and ionic exchange are simultaneously performed on the surface of used biosorbent, and the rate determining step was chemical bonding at the active cites of biosorbent (Coleman et al., 1956).

Table 2. Kinetic parameters for removal of Cd(II) ions using SA-Sc composite

	Pseudo-first-order kinetic			Pseudo-second-order kinetic		
	q_e (mg/g)	k_1 (mg/g·min)	R^2	q_e (mg/g)	$k_2 \left(\frac{g}{min \cdot mg}\right)$	R^2
1st cycle	3,217	0,1543	0,982	4,047	0,1184	0,9982
2nd cycle	2,756	0,05176	0,968	4,15	0,0585	0,9982
3rd cycle	2,109	0,04243	0,9772	3,956	0,0426	0,99984
4th cycle	2,013	0,03757	0,978	3,219	0,0327	0,9997

Conclusion

In this work, in order to produce advanced biosorbents for cadmium removal from aqueous solutions, non-pathogenicand safe *Sacharomyces cerevisiae* cell biomass was immobilized in silica-alginate composite material. SEM micrographs showed spherical composite particle with average size of 3 mm. Energy dispersive spectroscopy showed cadmium ions adsorbed on internal and external surface of biosorbent. The obtained composite displayed high cadmium removal efficiency of 99.2, 95.7, 88.3 and 78% in the successive four adsorption steps. The adsorption capacity after four step of Cd(II) removal was ~14,2 mg of Cd(II) per g of biosorbent. Pseudo-second-order kinetic agree well with the experimental values suggesting both adsorption and ionic exchange are simultaneously performed on the surface of used biosorbent, and the rate determining step was chemical bonding at the active cites of biosorbent.

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MEAT COLOUR DIFFERENCES BETWEEN ALPINE, BALKAN AND SERBIAN WHITE GOAT BREEDS SLAUGHTERED AT 18 KG OF BODY WEIGHT

Nikola Stanišić[1], Nevena Maksimović[1], Bogdan Cekić[1], Dragana Ružić-Muslić[1], Ivan Ćosić[1], Nemanja Lečić[1], Maja Petričević[1]

Abstract: Meat colour was evaluated in 36 goat kids of both genders equally from three breeds: Alpine, Balkan and Serbian white breed. Animals were slaughtered at 18 kg of body weight and three muscles were analysed for colour characteristics and pH value: *m. longissimus lumborum*, *m. psoas major* (tenderloin) and *m. semimembranosus*. pH values differ significantly only for *m. longissimus lumborum* muscle, whereas Balkan kids had a significantly higher pH value than Alpine and Serbian white ($p<0.001$). The goat breed significantly affected meat CIEL*a*b* values for all muscles analysed. Lightness (L*) was generally the highest for Balkan and lowest for the Alpine breed. The differences in redness (a*) and Chroma values (C*) were not significant, while the yellowness only differed for *m. longissimus lumborum* muscle, whereas Serbian white kids had higher b* values compared to Alpine kids. As a colour saturation characteristic, the hue angle was higher in meat from indigenous breeds (Balkan and Serbian white) than in the Alpine breed.

Keywords: meat colour, pH value, indigenious goat breed

Introduction

The total number of goats in the world is about 1 billion, of which a majority are in developing countries (Pophiwa et al., 2020). The lower amount of subcutaneous and intramuscular fat, favourable carcass composition and high meat quality are the main drivers behind the trend in increasing their overall number in the world (Stanišić et al., 2012). However, this increase is not worldwide significant due to its low economic significance compared to other animal breeds (Tshabalala et al., 2003). Goat meat also has more polyunsaturated fatty acids when compared with other ruminants, like cattle and sheep (Banskalieva et al., 2000). The popularity and acceptability of goat meat vary considerably between countries and ethnic groups. In South Europa and Latin America, meat from younger goats is popular, while meat from adult

[1] Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080 Belgrade, Serbia

goats is favoured in the Indian subcontinent (Naude and Hofmeyr, 1981). Countries like Greece, Spain, Italy and France are significant producers since they produce two-thirds of the total amount of goat meat produced in Europe (Ripoll et al., 2011).

In Serbia, goats are traditionally raised in a smallholder farming area with 1-2 animals per household, mainly in the mountainous. The production of goats in Serbia is mainly focused on dairy breeds. A significant number of goats in Serbia are from autochthonous breeds, such as Balkan and Serbian white goats. According to Žujović et al. (2011), the composition of the goat population is Balkan breed (47%), various hybrids (35%), Serbian white breed (15%) and Alpine (2–3%). Even though the main product obtained from goats in Serbia is milk, meat production should not be neglected.

Consumer evaluations and preferences of meat quality are primarily focused on freshness, colour, degree of marbling and texture. Meat colour is one of the most critical characteristics of meat as it influences consumer purchasing choices (Zervas and Tsiplakou, 2011). Meat which is more yellow and darker in colour is usually perceived to be obtained from older animals, so consumers mostly prefer pale or pink meat (Kosum et al., 2003). It is well known that meat colour is affected by many factors in the feeding–breeding system (Alcalde and Negueruela, 2001), of which animal breed, age, diet and meat cut are the most important ones (Priolo et al., 2001; Dhanda et al., 2003). Additionally, after slaughter, the muscle undergoes ageing, which changes the meat's colour, texture and flavour (Xiao et al., 2022).

To date, the information about the meat colour characteristics of indigenous Serbian goat breeds could be more extensive. Hence, this study was designed to provide valuable information regarding meat colour differences between two indigenous breeds (Balkan and Serbian white) and one noble goat breed (Alpine).

Materials and methods

All the animal management was conducted respecting animal welfare. The trial was carried out in the experimental farm of the Institute for Animal Husbandry (Belgrade, Serbia) on three groups of goat kids: Alpine, Balkan and Serbia white breed. Each group consisted of 12 kids (6 male and 6 female), reared under an intensive system and fed with grass hay and concentrate. Hay consisted of Festucetum vallesiacae grass mixture. The composition of the concentrate used in the feeding was as follows: proteins (16.0%), moisture (13.5%), fibre (8.0%), ash

(8.0%), calcium (0.8–1.0%), phosphorus (0.5–0.7%) and sodium (0.2–0.3%), with an addition of vitamins and minerals. Feeding was ad libitum until slaughter.

After reaching 18 kg of body weight, all animals were transported to the slaughterhouse. They were denied feed 12 h before slaughter, but they had free access to water. Animals were electrically stunned and slaughtered according to standard commercial procedures. After removing skin and head, front and rear legs and evisceration, carcasses were placed in cold storage at 4 °C for the next 24 hours. All meat samples (*longissimus lumborum*, *psoas major* and *semimembranosus*) were taken from the left side of the cooled carcasses.

The pH value of meat was measured by pH-meter Hanna, HI 83141 (Hanna Instruments, USA), equipped with a puncture electrode. Before measuring, the pH meter was calibrated using standard phosphate buffers (ISO 2917, 1999).

The colour of freshly cut meat surface, expressed in CIE L*a*b* colour coordinates, was measured after 30 min blooming time (samples were stored in contact with air at room temperature) using Chromameter CR-400 (Minolta Co. Ltd, Tokyo, Japan) with 8 mm aperture size, D65 illuminant and a 0° standard observer angle. Chromameter was calibrated using a white ceramic plate (Y=87.2, x=0.3173; y=0.3348; 0° observer). Three readings were done on non-overlapping areas of the muscles and the average value was used for data analysis. The a* and b* values were used to determine chroma = $(a^2 + b^2)^{1/2}$ and hue (°) = tan−1(b/a) according to Tapp et al. (2011).

In order to determine the effect of breed on the colour characteristics of meat, a single-factor analysis of variance was performed using SPSS 20.0 software (IBM SPSS Statistics, Version 20, IBM Corp, USA). If the effect of the breed was found significant, the LSD test was used to identify the significant differences between groups. All differences were considered significant at $p<0.05$ and data are presented as mean value ± standard deviation

Results and discussion

The final pH value of meat is an important indicator that significantly affects meat colour characteristics (Liu and Chen, 2000), as the pH of goat muscle declines significantly after slaughtering, affecting the overall meat quality (Hamoen et al., 2013). The differences in pH values between breeds per muscle are shown in Tables 1, 2 and 3. There were no significant differences among breeds within a muscle group ($p>0.05$), except for *longissimus lumborum*, where Balkan goats had a significantly higher pH value compared to Alpine and Serbian white (Table 2). Since higher meat pH can indicate pre-slaughter

stress, this difference could be explained by the different responses of Balkan goats to pre-slaughter handling (Dhanda et al., 2003). In the research of Bañón et al. (2006), Santos et al. (2007), Peña et al. (2009) and Ripoll et al. (2011), the final pH values for goat muscles were higher than 5.5, which is in agreement with this experiment. England et al. (2016) stated that the final meat pH value depends on the breed and muscle type of goats, which can explain the differences found for *longissimus lumborum* in this research (Table 2). Gawat et al. (2022) showed that the average pH values of raw goat meat ranged from 5.76 to 5.98, regardless of the breed.

Table 1. pH value and colorimetric parameters of *Longissimus lumborum* of kids

Parameters	Alpina	Balkan	Serbian white	p-value
pH	5.76 ± 0.14[a]	6.28 ± 0.10[b]	5.64 ± 0.17[a]	<0.001
L*	34.02 ± 2.13[a]	37.67 ± 1.88[ab]	35.80 ± 1.98[b]	0.002
a*	11.02 ± 2.60	9.20 ± 1.29	11.83 ± 0.79	0.099
b*	6.19 ± 1.55[a]	6.44 ± 1.26[ab]	7.26 ± 1.41[b]	0.030
h	29.37 ± 2.22[a]	35.08 ± 1.64[ab]	31,53 ± 1.86[b]	0.001
C*	12.65 ± 2.90	11.24 ± 2.79	13,89 ± 2.52	0.101

Table 2. pH value and colorimetric parameters of *Psoas major* of kids

Parameters	Alpina	Balkan	Serbian white	p-value
pH	6.15 ± 0.32	6.24 ± 0.44	6.07 ± 0.29	0.101
L*	34.29 ± 1.73[a]	38.60 ± 0.71[b]	35.08 ± 1.44[a]	<0.001
a*	15.40 ± 2.16	13.58 ± 1.15	15.07 ± 1.55	0.285
b*	7.59 ± 0.99	8.58 ± 0.67	8.19 ± 0.37	0.064
h	26,33 ± 1.31[a]	32.27 ± 1.01[b]	28.50 ± 1.43[b]	<0.001
C*	17.19 ± 2.32	16.07 ± 2.21	17.17 ± 1.74	0.668

Table 3. pH value and colorimetric parameters of *Semimebranosus* of kids

Parameters	Alpina	Balkan	Serbian white	p-value
pH	5.90 ± 0.43	6.07 ± 0.47	5.91 ± 0.33	0.098
L*	34.29 ± 0.99[a]	36.55 ± 1.88[b]	35.79 ± 2.42[ab]	0.016
a*	12.06 ± 1.75	11.70 ± 1.54	11.30 ± 1.87	0.299
b*	7.10 ± 1.16	6.74 ± 1.02	7.63 ± 0.96	0.115
h	30,47 ± 1.61[a]	30,09 ± 1.27[a]	34,21 ± 0.97[b]	<0.001
C*	14,00 ± 1.98	13,51 ± 1.62	13,64 ± 2.06	0.587

Meat lightness is a critical quality characteristic, as consumers prefer lighter meat and associate it with younger animals (Bañón et al., 2006). In the present trial, meat from Alpine kids was darker (lower L* values) compared to Balkan

kids, while Serbian white kids were intermediate (p=0.02) for *longissimus lumborum* (Table 1). For tenderloin (psoas major), L* values were highest for Balkan kids (38.60), with no significant differences between the two other breeds (Table 2). For the *semimembranosus* muscle, L* values were the lowest for Alpine kids (34.29) and highest for Balkan kids (36.55), while Serbian white was intermediate (Table 3). Lightness values of muscles reported in this study were lower than those reported by Santos et al. (2007), ranging from 44 to 50 in Bravia, Serrana and Bravia × Serrana breeds slaughtered at 8–11 kg. Similar values were reported by Bañón et al. (2006) for Murciano-Granadina kids meat, which had an average L* of 48.70 and Peña et al. (2009) for Criollo Cordobés and Anglonubian kids, where L* value ranged from 42.54 to 48.82. Older animals slaughtered at a higher final weight were probably the reason for the lower L* values found in this study (Peña et al., 2009). Dhanda et al. (1999) reported darker meat (L*<42) of Chevon goats. Regarding this, Marichal et al. (2003) and Argüello et al. (2005) reported a decrease of 2–4 points of L* value with an increase in slaughter weight from 6 kg to 10 kg. On the contrary, the effect of breed (Turkish Saanen, Gokceada and Malteseon goat slaughtered at 90 days of age) on lightness (L*) values of *longissimus dorsi* muscles was not significant in the research of Ekiz et al. (2010). Variations of L* values between genotypes can be explained by the differences in the meat fibre composition that influence different light scattering properties (Hughes et al., 2020). Overall, the lightness values were in the acceptable range of (L*) > 34 (Holloway and Wu, 2019).

A significant effect of genotype on a* values of goat meat was previously reported (Dhanda et al., 1999, 2003; Santos et al., 2007). The redness (a*) colour values of meat cuts were within the acceptable range from 9.5 to 19 (Holloway and Wu, 2019). In general, redness increases with the slaughter weight due to increasing the haem pigment of older animals (Lawrie, 1998). Altough, some authors found no significant differences in meat a* value with increasing the slaughter weight (Todaro et al., 2002; Peña et al., 2009). Additionally, the differences in redness values can be explained by the different muscle fibre compositions between breeds, which can influence myoglobin oxidation rate (Bakhsh et al., 2019). In the current study, the redness values of all three analyzed muscles are higher than those reported by Todaro et al. (2002) for kids slaughtered from 5 to 10 kg. However, other authors reported similar values (Dhanda et al., 2003; Santos et al., 2007). In the research of Gawat et al. (2022), meat from Boer crosses and feral goats differ in a* values for both *longissimus lumborum* and *semimembranosus* muscles, which also affected the value of

chroma (colour intensity) between genotypes and muscles. Dhanda et al. (2003) reported higher a* values for Australian feral goats than Boer crosses. Higher *longissimus lumborum* pH values of Balkan goats found in this research can be correlated to lower redness (McKeith et al., 2016; Zhang et al., 2018).

Goat slaughter weight did not affect meat yellowness (b*) value in the research of Peña et al. (2009), although b* decreases with a significant increase in animal slaughter weight in the research of Dhanda et al. (1999). In the present research, b* values significantly differ (p=0.030) between breeds only for *longissimus lumborum* muscle, where the highest values were found for the Serbian white breed (7.26) and lowest for the Alpine breed (6.19), while the Balkan breed was intermediate (Table 1).

Hue angle (h), as a colour saturation parameter, gives a more realistic overview of meat colour compared to single indicators, such as a* and b* and it is often used to describe meat discolouration (Emami et al., 2015). Therefore, the higher h value results from a decrease in a* relative to b* value (Luciano et al., 2009). In the present research, the higher h values of meat from indigenous breeds (Balkan and Serbian white) compared to the Alpine breed shows that a lighter colour characterizes this meat. These results agree with the findings of Dhanda et al. (2003), Marichal et al. (2003) and Argüello et al. (2005) but were much lower than those reported by Todaro et al. (2002) and Ripoll et al. (2011). Interestingly, Ekiz et al. (2010) found no significant differences in hue values of *longissimus dorsi* muscles between three goat breeds (Turkish Saanen, Gokceada and Malteseon).

Chroma value (C*) is correlated to the pigment content, and high values indicate a more vivid colour and denote a lack of greyness (Miltenburg et al., 1992). Todaro et al. (2002), Argüello et al. (2005) and Peña et al. (2009) found no differences in meat chroma values between goats slaughtered at different weights. Ripoll et al. (2011) found that chroma values were strongly correlated to yellowness (r=0.95; p<0.001). However, in the current research, there were no significant differences in C* values between breeds for all muscles analyzed. These results are not in agreement with the research of Ekiz et al. (2010), where authors found a significant difference in *longissimus dorsi* C* values between goat breeds, although the values were similar to those reported in this trial (Table 1).

Conclusion

The results obtained in this study demonstrated the significant effect of goat genotype on pH and meat colour characteristics for all three analyzed muscles,

whereas the biggest effect was established for l*ongissimus lumborum* muscle. Balkan kids had a significantly higher pH value compared to Alpino and Serbian white, which probably influenced Balkan kids' significantly higher meat lightness than Alpino. Yellowness only differed for *longissimus lumborum* muscle, whereas Serbian white kids had a higher b* value than Alpino kids. As a colour saturation characteristic, the hue angle was higher in meat from indigenous breeds (Balkan and Serbian white) than in the Alpino breed. Interestingly, all three breeds had similar redness (a*) and Chroma values (C*).

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MICROBIOLOGICAL ASSESSMENT OF ICE CREAM SOLD AT THE TERRITORY OF BELGRADE

Radoslava Savić Radovanović[1], Slobodanka Janićijević[2], Jelena Aleksić Radojković[1]

Abstract: The aim of this research was to evaluate the microbiological quality of ice cream sold at catering facilities-restaurants, cake shops and bakeries on the territory of Belgrade. The material consisted of 40 samples of ice cream collected during monitoring in 2021 and 2022. Microbiological analyses were carried out according to the Rulebook on General and Special Conditions of Food Hygiene at any Stage of Production, Processing and Trade ("*Official Gazette of the RS*", No. 72/2010, 62/2018). ISO standard methods for detection of microorganisms in ice cream samples were applied. Out of 40 samples, all were in accordance to the safety criteria, as Salmonella spp. were not detected in any of tested samples. Four samples (10%) did not meet the hygiene criteria in production processas more than 100 CFU/g Enterobacteriaceae were detected. Out of 4 samples, one sample of ice cream was produced in the small craft facility and 3 samples were from mobile ice cream vending machines. It can be concluded that examined samples of ice cream from catering facilities in the area of Belgrade were safe for consuption, since they met the safety criteria prescribed by the applicable legislative. The improvement of hygienic measures during the production process of ice cream in small craft fecilities and vendom mobile mashines was ordered.

Keywords: Enterobaceteriacae, ice cream, microbiology, safety, *Salmonellae* spp.

Introduction

Ice cream is a frozen product of milk and according to the legislative (Rulebook of Quaility of Milk Products and Starter Cultures, *"Official Gazette RS No 33/10, 69,10, 43,13, 34/14)* belongs to the group of frozen desserts. It contains not less than 2.5% milk and/or vegetable fat in dry matterand not less than 24% in total

[1]University of Belgrade, Faculty of Veterinary Medicine, Bulevaroslobođenja 18, Belgrade, Serbia (mimica@vet.bg.ac.rs)
[2]Ministry of Agriculture, Forestry, Water Economy of Serbia, Omladinskih brigada 1, Belgrade, Serbia

dry matter, and must contain milk and/or vegetable protein.The technological process of ice cream production can be divided into two phases: preparation of the ice cream mixture (mix) and freezing of the ice cream mixture. The production of ice cream includes totally eight steps (raw compounds, preparation of ice cream mixture, pasterization and homogenization, ripening of mixture, freezing, packing, hardening and storage). Any ingredient used in the production of ice cream may represent a source of microorganisms that can affect the composition of the microbiota in the finished product (Kambamanoli-Dimou, 2014). Pasteurization, freezing and hardening are the main steps to eliminate the microbiological hazards. Ice cream can be sold in soft or hard consistensy.It is a very popular dessert which is usually consumed during summer months in all age groups of consumers, especially in children (Mokbul et al., 2016).This dessert has a history that stretches across the globe, with different countries enjoying various versions such as *kulfi* in India, *gelato* in Italy, and *mocha* in Japan. It seems every country has its own spin on this delicious frozen product. New Zealand leads the world in ice cream consumption with a per capita consumption of 28.4 liters per year. This country is renowned for the high standard of its dairy products. Folowing, next on the list of highest cosumers of ice cream in the world in 2020 were: United States of America (20.8 L/capita), Australia (18.0 L/capita), Finland (14.2 L/capita), Sweeden (12.0 L/capita), Canada (10.6 L/capita), Denmark (9.8 L/capita), Ireland (8.4 L/capita), Italy (8.0 L/capita), United Kingdom (7.0 L/capita).The market demand is still considerable, as evidenced by the amounts of production, which reached over 2.9 billion liters in the E.U. and almost 5 billion liters in the U.S. in 2020 (Eurostat, 2022; Economic Research Service, 2022).

The production of ice cream in the Republic of Serbia has been growing in recent years, and according to the data of the Serbian Chamber of Commerce (SCC), a particularly large growth has been recorded since 2019, when 51 percent more ice cream was produced than a year earlier.According to data from Statistical Office of the Republic of Serbia in 2021, 426.744hL of ice cream(including sherbet and lollipops, excluding mixtures and ingredients for ice cream)were produced, and 416.346 hL were sold.In addition to the large ice cream factories, which produce the largest quantities, ice cream is produced by round a hundred small craft producers. Considering all this, the production of ice cream is important issue in the food industry. Since this product isa very popular ultimate old-fashioned treat, that is readily consumed nowadays, we decided to evaluate the microbiological quality of ice creams sold at the territory of Belgrade.

Materials and methods

The material represented 40 samples of ice creams collected during monitoring in summer 2021 and autumn 2022 at catering facilities-restaurants, cake shops and bakeries at the territory of Belgrade city.Samples were collected aseptically in sterile plastic jars, labelled and transported in frozen condition, in transport freege to the laboratory where bacteriological analyses were started immediately.

Microbiological analyses were performed according to the Rulebook on General and Special Conditions of Food Hygiene at any Stage of Production, Processing and Trade *("Official Gazette of RS"*, No. 72/2010, 62/2018). For detection of *Salmonella* spp. in ice cream samples SRPS EN ISO 6579-1: 2017 method was used.The SRPS ISO 21528-2: 2017 method was used to detect Enterobacteriaceae in aim to examine the hygiene criteria of ice creamproduction process.

Results and discussion

The obtained results showed that out of 40 samples from catering facilities, 4 (10%) did not meet the hygiene criteria of the production process, as the presence of Enterobacteriaceae greater than 100 CFU/g was detected in 4 samples (1 sample from a craft facility and 3 samples from mobile ice cream vending machines).In none of 40 samples was not detected the presence of *Salmonellae* spp.

Table 1. Results of bacteriologycal analysis of ice cream samples collected in the catering facilities at territory of Belgrade city

Year	*Sallmonellae* spp. in 25 g				Enterobacteriacae log CFU/ ml			
	positive		negative		positive		negative	
	Number	%	Number	%	Number	%	Number	%
2021	0	0	25	62.5	4	10	21	52.5
2022	0	0	15	37.5	0	0	15	37.5
Total	0	0	40	100	4	10	36	90

Ice cream is a complex food matrix and a good medium for growth of microorganisms due its high content of nutrient constituents, nearly neutral pH (6–7) (Nalbone et.al., 2022; Kanbakan et al., 2004) and long storage duration (Bell and Kyriakides, 1998) as well. It consists of a frozen multiphase mixture

containing ice crystals, air bubbles and partially coalesced fat globules within an unfrozen serum phase of dissolved proteins, sugars and mineral salts (Nalbone etal., 2022). Due to that, this food may be classified as a high risk potential hazard which has been implicated in outbreaks of food poisoning (Baraheem et al., 2007). Microbial contamination can be introduced at various stages of production line from different human and environmental sources (Chukuezi, 2010). Heat treatment by pasteurization destroys most of the pathogens that pose risk to public health. However, what makes ice cream worthy of attention from a microbiological point of view is the significant amount of processing carried out after the pasteurization step (Cook and Hartel, 2010). This raises great concern especially for foodborne pathogens, which can contaminate the ice cream after heat treatment from the working environments or through the addition of contaminated ingredients. Overall, only a few outbreaks of foodborne illness related to ice cream consumption have been recorded and these are linked to different pathogens, such as Salmonella spp., *Listeria monocytogenes*, verotoxin-producing *Escherichia coli* and coagulase-positive staphylococci (Hennessyet al., 1996; Seo, 2006; Pouillot et al., 2015; De Schrijveret al., 2008; Fetschet al., 2014). Among these bacteria, the most severe outbreak was related to *Salmonella* Enteritidis, involving 224.000 cases of infections in the U.S., as the result of cross-contamination during the transport of a pasteurized mix into inadequately sanitized tanks in which contaminated liquid eggs were previously transported (Hennessy, 1996).

In the manufacturing processes, pasteurization was effective in destroying of pathogenic bacteria and freezing and hardening processes inhibited the microbial growth. Automatic machines that are widely used for ice-cream production in dairy industry minimize direct hand manipulation and possibility of cross contamination. Furthermore, low temperature (< 7°C) of ice-cream mix is unfavorable for the multiplication of bacteria.

Our results disagree from those of Mohammed et al. (2013) who examined 60 samples of ice cream and isolated *Salomonella* spp. in 6.6% samples from unpacked-street vendors. They identified *S.* Typhimurium and *S. paratyphi* B. These authors detected Enterobacteriaceae strains in 60% and 80% samples of ice cream from unpacked-shop and unpacked-street vendors respectively, which are much higher values than ours.Our results are similar to Zadre et al. (2010) who examined samples of ice cream in Dubrovnik city area during three calendar years and did not detect *Salmonella* spp. in any of samples. They detected Enterobacteriaceae in 2006 (81 samples/9.87%), 2007 (101/12.87%) and 2008 (74/8.1%), which is in accordance to our results.Higher levels of

Enterobacteriacae findings were reported by Fadihl et al. (2019), who out of 70 samples of ice cream detected in 28%. Our results are not in accordance to El-Makarem (2017) who analysed 100 (50 packaged and 50 unpackaged) ice creams and detected high numbers of Enterobacetriacae in 27 % of packaged ($2.1x10^3 \pm 0.8x10^3$ CFU/ml) and 48% of unpackaged ice cream samples ($1.9x10^4 \pm 0.8x10^4$ CFU/ml). Yan et al. (2022) reported that positive rate of *Salmonellae* spp. in ice cream was very low at 0.10% among 2887 samples of ice cream from defferent regions in China. Güçlü et al. (2022) detected Enterobacteriacea out of 75 samples of ice cream in Turkey at the level of 40% (n=10) in plain ice creams (1.62×10^4 CFU/g), 12% (n=3) in fruits (1.13×10^4 CFU/g) and 40% (n=10) in nuts ice cream (7.3×10^3 CFU/g) which are much higher values than we reported. Also, according to these authors *Salmonella* was not found in any of 75 ice cream samples. The Food Inspection Agency's 2017-2020 routine monitoring programs found no *Salmonellae* spp. in 1186 samples of ice cream collected from retail locations in 11 cities across Canada which is accordance to our results. Adžić (2022) reported that out of 35 samples of ice cream colected in café pastry shops at Novi Sad, 5.71% were positive for Enterobacteriacae and none for *Salmonellae* spp. Finally, results of Milutinović (2016) showed that all ice cream samples collected in Niš were free of *Salmonella* spp. and Enterobacteriacae.

Conclusion

Ice cream is one of the many dairy products consumed around the world. During the summer season, consumption increases and can pose a risk to consumers due to high temperatures and lack of hygiene. The potential microbiological hazards in the final products may be introduced after pasteurization due to the addition of contaminated ingredients and improper handling procedures. The above study showed that the overall risk associated with the consumption of ice cream in the Belgrade area is low, as *Salmonellae* spp. were not detected in any of 40 samples tested. Our results showed that out of 40 ice cream samples, Enterobacteriacae were detected in 4 (10%), indicating low hygiene during the production process. In aim toimprove the microbiological quality of ice cream hygiene measures were ordered. It was also indicated that regular controls should be performed on ice cream produced in vending machines due to inadequate manufacturing practices and poor hygiene of the working environment.

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INSTRUMENTAL COLOUR AND TEXTURE PROPERTIES OF FRANKFURTER-TYPE SAUSAGES WITH PLANT OILS

Slaviša Stajić[1], Vladimir Kurćubić[2], Vladimir Tomović[3], Dušan Živković[1]

Abstract: The aim of this research was to examine the influence of partial and total replacement of pork backfat with two quite different (in terms of sensory properties) plant oils (grapeseed and pumpkinseed oil) on instrumental colour and texture of frankfurters. The influence on colour was more pronounced when pumpkinseed oil emulsion was used as backfat replacer – these frankfurters were less red and more yellow than control and frankfurters with grapeseed oil emulsion. The effect was more pronounced with the increase of backfat replacement degree. No significant influence on instrumental texture parameters was observed.

Keywords: frankfurters, grapeseed oil, pumpkinseed oil, colour, texture

Introduction

Meat products lost their primary function in the last 50–60 years – to preserve meat and provide energy, proteins of high biological value and minerals, so thay are nowadays highly valued mainly because of their sensory properties. However, some components of meat products such salt, phosphates, nitrites, fat, saturated fatty acids etc., are mentioned as having a negative impact on human health. High daily intake of animal fat rich in saturated fatty acids (SFA) is associated with a higher risk of cardiovascular diseases (McAfee et al., 2010).

Emulsion-type meat products such as hot-dogs and frankfurters are well-known worldwide and together with burgers/patties represent a hallmark of the fast-food industry. Total fat content in emulsion-type meat products is usually within interval 20–30% (Pisinov et al., 2021). Fat is very important because it contributes to the physical and chemical stability of the product – fat stabilizes the meat emulsion, reduces cooking loss, increases the ability to retain water, and greatly affects the colour and juiciness (Câmara and Pollonio, 2015).

[1]University of Belgrade, Faculty of Agriculture, Nemanjina 6, Belgarde, Serbia (stajic@agrif.bg.ac.rs)
[21]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia
[3]University of Novi Sad, Faculty of Technology, Bulevar Cara Lazara 1, Novi Sad Serbia

Reduction of fat content significantly altered technological and sensory properties of emulsion-type meat products (Cengiz and Gokoglu, 2007). In view of this, only a partial fat reduction and/or replacement can be done. The two strategies have been established to achieve this (Kurćubić et al., 2022): i) partial decrease of fatty tissue and/or replacement with non-lipid replacers and ii) partial fatty tissue replacement with oils rich in polyunsaturated fatty acids (PUFA).

Regarding emulsion-type meat products several research studies were successfully conducted with the aim of replacing fatty tissue with non-lipid replacers (Bajcic et al., 2023; Kurćubić et al., 2020). Also, fatty tissue was partially replaced with oils to obtain a favourable fatty acids profile (Stajić et al., 2020; Stajić et al., 2018). The research data indicate that the impact of partial fatty tissue replacement with oils on properties of meat products depends on oil properties, level of replacement, oil stabilization procedure and type of meat product (Stajić and Živković, 2021). While refined grapeseed oil is very light and has a neutral odour and taste, pumpkin oil is darker and has a specific odour and taste (Stajić et al., 2020; Stajić et al., 2014). Firstly, oils were stabilized in emulsion with soy protein isolates (commonly used ingredient in meat processing). Later, gel emulsions, double emulsions, oleogels etc. were used for oil stabilization (Stajić and Vasilev, 2022).

The aim of this research, which is a follow-up on the research conducted by Stajić and Živković (2021), was to examine the influence of partial and total replacement of pork backfat with two plant oils (grapeseed and pumpkinseed oil), quite different in sensory properties, on instrumental colour and texture of frankfurters.

Materials and methods

Frankfurters were prepared in the same manner as described by Stajić and Živković (2021). Control treatment (CON) was prepared with beef, pork backfat and ice in the ratio of 50:25:25. Six modified treatments were prepared by replacing 20%, 60% and 100% of backfat with emulsions of grapeseed oil (GSO20, GSO60 and GSO100, respectively) and pumpkinseed oil (PSO20, PSO60 and PSO100, respectively). All treatments were prepared in the same manner. Beef and backfat were initially ground trough a 10 mm plate. Afterwards, beef was mixed and ground with ice, salt and phosphates in the cutter (knife and bowl speed 1,410 and 12 rpm, respectively) for 2 min. Then, pork backfat, oil emulsion (for modified treatments) and other ingredients were added and emulsified (knife

and bowl speed 2,780 and 24 rpm, respectively) until the temperature of 14 °C was achieved. Frankfurters were stuffed into collagen casings, (diameter 22 mm and 50 g approximate weight) and underwent the following regime in a smoking/cooking chamber: drying 10 min at 50 °C, smoking 30 min at 60 °C, and heated at 85°C until the temperature in the center of the product reached 70 °C. Afterwards, the frankfurters were showered with warm water (about 50 °C) for 15 min and cooled in a cooling chamber at 3±1 °C overnight. Emulsions of vegetable oils were prepared immediately before frankfurter preparation in the manner described by Stajić et al. (2014): 5 parts of water were mixed with one part of soy protein isolate (SUPRO EX 33 IP, Solae, LLC) in a cutter for two minutes, then five parts of cooled oil was added and mixing was continued for 3 to 5 minutes until the emulsion became compact and stable.

The CIE L*a*b* colour coordinates were determined by MINOLTA Chroma Meter CR-400 (Minolta Co., Ltd., Osaka, Japan) using an 8-mm aperture size, illuminant D65 and a 2° standard observer angle. The Chroma Meter was calibrated using a Minolta calibration plate (No. 11333090; Y = 92.9, x = 0.3159; y = 0.3322). C* (chroma) and h (hue angle) were calculated using software Konica Minolta Color Data Software CM-S100w Spectra Magictm NX Pro QC ver. 2.0. Two measurements on four frankfurters of each treatment were taken (n=8).

Total colour difference (TCD) relative to CON was calculated using the standard equation:

$$\mathrm{TCD} = \sqrt{(L^*_{MT} - L^*_{CON})^2 + (a^*_{MT} - a^*_{CON})^2 + (b^*_{MT} - b^*_{CON})^2}$$

MT – modified treatments; CON – control

Texture profile analysis was performed using the universal texture analyser TAXP (Stable Micro System, Godalming, UK). Samples for texture analysis were cylindrical, 2 cm in height and original diameter. The samples were compressed twice to 40% of their original height, with a compression aluminium platen of 75 mm (P/75) and constant test speed of 1 mm s^{-1}. Hardness, springiness, cohesiveness and chewiness were evaluated and were obtained using the available computer software. Eight readings were taken for each treatment (four frankfurters with two samples from each).

One-way ANOVA was performed for testing the influence of backfat replacement. Tukey's HSD test was used to identify significant ($p < 0.05$) differences between treatments. Statistical analysis was performed with software Statistica 12.5 PL, for Windows (StatSoft, Inc., Tulsa, OK, USA).

Results and discussion

The replacement of backfat with oil emulsions had a significant influence on all parameters of instrumental colour (Table 1). The effects are dependent on replacement degree and oil type. Lightness (L* values) was more altered when backfat has been replaced with grapeseed oil emulsion. Also, progressive increase of L* values was observed with the increasing content of grapeseed oil emulsion. However, no significant difference ($p > 0.05$) was observed between GSO60 and GSO100. This was not observed when pumpkinseed oil emulsion was used as backfat replacer – PSO60 and PSO100 had similar L* values to control ($p > 0.05$).

Table 1. Results of instrumental colour analysis of cross section

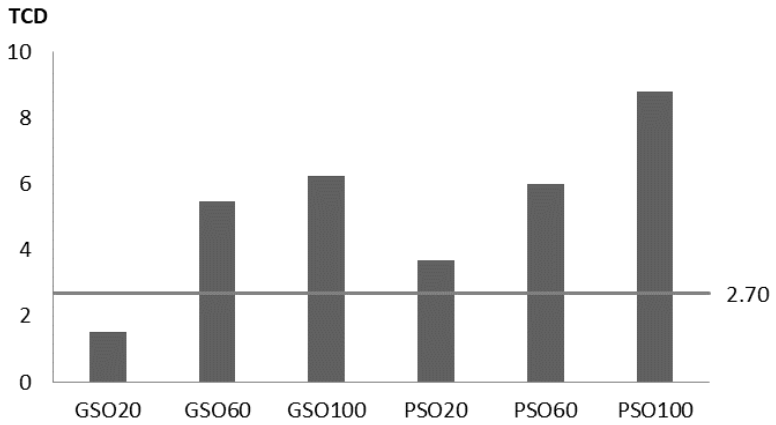
	L*	a*	b*	C*	h
CON	62.62±0.60c	16.71±0.45a	13.65±0.22d	21.55±0.44b	39.27±0.54f
GSO20	63.91±0.40b	16.40±0.30a	13.99±0.30d	21.55±0.39b	40.50±0.47f
GSO60	67.86±0.48a	15.12±0.33b	13.92±0.20d	20.54±0.33c	42.69±0.51e
GSO100	68.37±0.67a	14.33±0.37c	13.93±0.33d	19.99±0.22c	44.20±1.25d
PSO20	64.63±0.70b	14.64±0.61bc	15.77±0.27c	21.53±0.29b	47.20±1.61c
PSO60	62.81±0.52c	12.62±0.58d	17.91±0.27b	21.89±0.48b	54.91±1.10b
PSO100	62.87±0.44c	10.75±0.39e	20.07±0.37a	22.77±0.44a	61.89±0.77a

CON – control treatment; CON – control treatment; GSO20 & PSO20 – 20% backfat replaced with grapeseed oil emulsion and pumpkinseed oil emulsion respectively; GSO60 & PSO60 – backfat replaced with grapeseed oil emulsion and pumpkinseed oil emulsion respectively; GSO100 & PSO100 – 100% backfat replaced with grapeseed oil emulsion and pumpkinseed oil emulsion respectively; a–f Values (mean±SD) in the same column with different letters are significantly different ($p<0.05$)

Regarding redness (a* values), only GSO20 had similar values to CON, while in other treatments a* values were progressively and significantly lower with the increase of backfat substitution level. Yellow tones (b* and h values) progressively and significantly ($p < 0.05$) increased with pumpkinseed oil emulsion content. In GSO treatments b* values were similar ($p > 0.05$) to CON, while hue angle values progressively increase with grapeseed oil emulsion level. However, the effect was more pronounced in frankfurters with pumpkinseed oil emulsion as backfat replacer.

Chroma values had the opposite trend regarding the oil added – they decreased when grapeseed oil emulsion was used and increased when pumpkinseed oil emulsion was used while significant differences were

observed in treatments with total backfat replacement with oil emulsions. These very intense changes of colour properties in frankfurters with pumpkinseed oil emulsions could influence sensory acceptability which was confirmed in a previous research by Stajić and Živković (2021) – frankfurters with 60% and 100% backfat replacement had significantly lower grades regarding colour compared to control and all GSO treatments. On the other hand, all GSO treatments received similar grades to control treatment. Stajić et al. (2020) also reported significantly higher b* and h* values when 25% of backfat was replaced with encapsulated pumpkinseed oil. Regarding the use of grapeseed oil, according to Stajić et al. (2014), the influence on colour properties is more dependent on the oil stabilization procedure than oil colour properties.



Graph 1. TCD values of modified frankfurter compared to CON; Horizontal line indicates potential visual detection limit

Total colour difference (TCD) represents the quantification of the overall difference between two colours – e.g. modified treatments vs CON. Research indicates that TCD values less than 2.7 were probably not perceptible by consumers. TCD values (Graph 1) of all modified frankfurters, except GSO20 were higher than 2.7 and progressively increased with oil emulsion content. The effect was more pronounced when pumpkinseed oil emulsion was used as backfat replacer.

In general, it could be said that backfat replacement with oil emulsion led to lower hardness and chewiness. However, all parameters of instrumental texture were not significantly affected by the level of backfat replacement (Table 2).

Table 2. Results of texture profile analysis (TPA)

	Hardness (g)	Springiness	Cohesiveness	Chewiness (g)
CON	4537.27±976.20a	0.83±0.08a	0.84±0.02a	3203.67±897.81a
GSO20	3921.05±479.15a	0.83±0.04a	0.81±0.02a	2660.28±382.56a
GSO60	3873.99±492.63a	0.81±0.08a	0.82±0.01a	2586.96±401.82a
GSO100	3802.09±661.68a	0.86±0.06a	0.83±0.02a	2711.95±473.83a
PSO20	4039.64±570.42a	0.84±0.05a	0.80±0.01a	2720.33±505.02a
PSO60	4041.08±204.82a	0.83±0.04a	0.83±0.02a	2780.41±189.54a
PSO100	4217.14±697.53a	0.86±0.07a	0.81±0.07a	2920.98±508.96a

CON – control treatment; GSO20 & PSO20 – 20% backfat replaced with grapeseed oil emulsion and pumpkinseed oil emulsion respectively; GSO60 & PSO60 – backfat replaced with grapeseed oil emulsion and pumpkinseed oil emulsion respectively; GSO100 & PSO100 – 100% backfat replaced with grapeseed oil emulsion and pumpkinseed oil emulsion respectively; Values (mean±SD) in the same column with different letters are significantly different ($p<0.05$)

Conclusion

The results of this research indicate that oil properties should not be ignored in the creation of emulsion-type meat products with improved nutritional properties. In order to reduce the impact of emulsion-type meat products on colour, the level of backfat replacement should be adjusted to colour properties of the oil added.

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NATURAL ANTIMICROBIAL AGENTS: APPLICATION IN FOOD PRESERVATION AND FOOD BORN DISEASE CONTROL

Vladimir Kurćubić[1], Slaviša Stajić[2], Nemanja Miletić[1], Marko Petković[1], Igor Đurović[1], Vesna Milovanović[1]*

Abstract: Natural antimicrobial agents in food have gained much attention by the consumers and the food industry. The misuse of antibiotics has resulted in the dramatic rise of microorganisms that are antibiotic resistant and tolerant to several food processing and preservation methods. Additionally, increasing consumers' awareness of the negative impact of synthetic preservatives on health compared to the benefits of natural additives has caused interest among researchers in the development and usage of natural products in foods. This article reviews natural antimicrobial agents and their application in food preservation and food born disease control.

Keywords: natural antimicrobial agents, natural additives,food preservation, food born disease

Introduction

Food products are highly sensitive to microbial contamination that affect their quality characteristics and reduce their nutritional value. Moreover, the presence of microbial toxins or pathogenic microorganisms such as *Salmonella*spp., *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Campylobacter*, *Clostridium perfringens*, and *Aspergillus niger* may endanger consumer safety and cause foodborn diseases - FBD (Nummer et al., 2012).Recent data indicate the existence of more than 1,340 registered plants from which more than 30,000 compounds exhibiting antimicrobial (AM) effects have been extracted (Hayek et al., 2013). The use of AM agents of natural origin is widely accepted by consumers, because it has GRAS status (GRAS - Generally Recognized As Safe). Today, isolates of *Listeria monocytogenes*, *Clostridium perfringens*, *Salmonella* spp. and *Escherichia coli* are considered the

[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia.Corresponding author: (milovanovic.vesna@kg.ac.rs)
[2]University of Belgrade, Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade, Serbia

main causes of poisoning when consuming meat products (Ceruso et al., 2020; Cetin et al., 2020; Chang et al., 2020; Park et al., 2021).AM agents have various activities on different microorganisms due to their diverse physiologies, integrated either directly into food or to the packaging material where it is distributed over a period of time to maintain the safety and quality of the products, resulting in extended shelf life. The worldwide spread of pathogen-resistant bacteria threatens the public health of the population. Currently, infections caused by Gram-negative (Gr-) bacteria occur more often than Gram-positive (Gr+) bacteria in clinics. A report from the China Antimicrobial Resistance Surveillance System (CARSS) shows that Gr- bacteria account for 71.1% of 3.249.123 clinically isolated strains and the prevalence of Gr+ bacteria is 28.9% (Song et al., 2022).The increasing occurrence of dangerous infections caused by bacteria that are resistant to antibiotics of the latest generations (multidrug resistant - MDR) has made the research of new molecules in the field of medicine current on a global level.

Natural antimicrobial agents for food preservation

Compared to chemically synthesized substances, plants provide greater structural diversity and offer more opportunities for the identification of new AM compounds. Plants show excellent antibacterial effect due to their safety, efficacy, antimicrobial synergism and reduced drug resistance. The combination of herbs and chemical AM agents (of synthetic origin) for the treatment of infectious diseases is popular in clinical practice in China because of their synergistic or potentiating effects. Characterization of microorganisms can be very helpful for the selection of an AM agent such as cell wall composition – Gram-negative and Gram-positive, oxygen requirements- aerobes and anaerobes, growth stage - spores and vegetative cells, acid/osmosis resistance, optimal growth temperatures - mesophilic, thermophilic, psychotropic (Malhotra et al., 2015).

Alhough synthetic preservatives, nitrite and sulfites, proved to be highly effective against a broad range of pathogenic microorganisms in foods, their potential negative impact on human health has prompted the usage of naturally occurring antimicrobials to inhibit the growth of pathogens and prevent foodborn illness (Abdollahzadeh et al.,2014;Ahmedet al., 2014; de Oliveira et al.,2015). Natural AM agents can be obtained from various sources including plants, animals, bacteria, algae and fungi. Several studies related to plant antimicrobials have proved the efficacy of plant derivatives in food applications(Tajkarimi et al., 2010; Gyawali and Ibrahim, 2012). Natural plant

components can be obtained from fruits and vegetables (onion, garlic, cabbage, pepper, xoconostle, and guava), seeds and leaves (olive leaves, parsley, caraway, nutmeg, fennel, and grape seeds), and herbs and spices (oregano, thyme, marjoram, basil, rosemary, sage, clove, and cardamom)(Tajkarimi et al., 2010). AM phytochemicals can be divided into several categories such as: phenolics and polyphenols; quinones; flavones, flavonoids, and flavonols; tannins; coumarins; terpenoids and essential oils; alkaloids; lectins and polypeptides (Cowan, 1999). Allyl-isothiocyanate is the mainAM component of mustard and horseradish oil which exhibitAM activity against Gram-negative bacteria with lesseffect on lactic acid bacteria (Delaquis and Mazza, 1995).

Accordingly, food business entities began to evaluate the use of environmentally friendly and consumer-friendly additives, of natural origin, instead of synthetic additives (Ryu and Lee, 2018; Câmara et al., 2020).

Lee et al. (2020) sublimated observations, suggestions and guidelines for researching the possibility of using antioxidants in the meat industry. The use of synthetic antioxidants is considered more cost-effective, safer and simpler, thus reducing the use of natural antioxidants (Pokorný, 2007; Mbah et al., 2019).

Essential oils(EOs)are considered as a natural AMremedyfor FBDinstead of using synthetic agents. The essential oils are well known for its AM and antioxidant (AOX) properties due to the presence of phenolic functional group (Vergis et al., 2013). Herbal extracts have already been used to control diseases that occur as a result of food poisoning and food preservation (Mostafa et al., 2018).Flavonoids are the most promising AM agents, showing favorable antibacterial activity. Most alkaloids exhibit a relatively weak antibacterial effect; however, berberine shows strong AM activity. Many terpenes and partial EOs show strong AM activity (Liang et al., 2022).Our experiences indicate that in the future it is necessary to improve the investigation of the use of plant EOs and extracts as AM agents, as well as the research of new approaches, such as the application of a small dose of a synergistic combination of plant extracts (Kurćubić et al., 2012a; 2012b; Kurćubić et al., 2014; Kurćubić et al., 2015).

By-products of different fruits and vegetables are potentially good sources of phenolics that have a broad range of AM properties (Chanda et al., 2010).

Natural agents of animal origin such as lactoferrin, chitosan, milk-derived peptides, lysozyme and many other present potential antimicrobials which could be used as food additives (Gyawali and Ibrahim, 2012).

Lactoferrin,iron-binding glycoproteinin milk, possess AM activity against a broad range of bacteria and viruses(Lönnerdal, 2011). The AM properties of Lactoferrin against FBD microorganisms including *Carnobacterium*, *L.*

monocytogenes, *E. coli*, and *Klebsiella* have beenreported (Al-Nabulsi and Holley, 2005; Murdock et al., 2007).

Chitosan, as a polycationic biopolymer naturally present in the exoskeletons of crustaceans and arthropods, has received considerable interest for commercial applications in food(Tikhonov et al., 2006).

Lysozyme, as an enzyme that is naturally present ineggs and mammalian milk, exerts antimicrobial activity against microorganisms, especially Gram-positive bacteria by hydrolyzing 1,4-β-bonds in the bacterial cell walls. Thus, this enzyme could be utilized in medical, pharmaceutical, and particularly in the food industry as a preservative in various products, such as fruits and vegetables, meat, milk, and dairy products (Khorshidian et al., 2022).

Milk-derived peptides such as casein and whey proteins have been reported to possesswide rangebioactivitiesincluding AM activity. These peptides exhibit activity against a broad range of pathogenic microorganisms such as *E. coli*, *Helicobacter*, *Salmonella*, *Listeria*,*Staphylococcus*, yeasts, and filamentous fungi (Fadaei, 2012).

Antimicrobials of bacterial origin such as bacteriocin (produced by *Lactococus lactis*) and reuterin (produced by *Lactobacillus reuteri*) present widely recognized natural preservatives (Arqués et al., 2011; Bian et al., 2011).

Algae and mushrooms present natural sources of bioactive compounds that have a wide range of biological properties, such as AM, AOX, anti-inflammatory, cytotoxic, and other health promoting benefits.Algae and fungi can be consumed as dietary supplements in the form of capsules or tablets containing purified fungal or algae extracts and directlyused can be treated as a type of functional food(Ślusarczyk et al., 2021). The AM activity of different types of algae against pathogenic bacteria has been identified by several scientists as potential AM agents that may be useful in the food industry. AM activity of algae (*Himanthalia elongate*) and microalgae (*Synechocystis* spp.) against *E. coli* and *S. aureus* has been reported (Plaza et al., 2010).

The application of bioactive substances of plant origin is not always under the control of doctors, so the question of the safety of their use is open, because it can lead to the absence of biological or toxic effects (Rates, 2001; Kurćubić et al., 2022a, 2022b).In order to inhibit the growth of undesirable microorganisms and reduce lipid oxidation in meat products, bioactive compounds that exhibit strong AM and AOX power can be incorporated into the product formulation, coated on its surface or incorporated into the packaging material for packaging the product (Horita et al., 2018; Nikmaram et al., 2018).

Conclusion

The basis of progress lies in innovation and competitiveness, and the benefits of implementing the aforementioned "bioinitiatives" are, in addition to preserving the environment, improving the quality of existing foods, creating new, healthier or functional products with desirable techno-functional properties, enriched with the highest level of bioactive substances or reformulated, with positive repercussions on people's health.One of the unsolved challenges for many phytochemicals is tracing effective routes and forms of administration that can release the active antimicrobial compound at the target site during systemic infections. The selection of compounds responsible for antimicrobial activity in complex mixtures such as extracts and essential oils and their potential pharmacological interactions is also a puzzle. For this purpose, it is necessary to use modern technologies, antimicrobial tests with internationally recognized standardized protocols and the use of plant material with appropriate quality controls.

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MICROCONTROLLER CONTROL SYSTEM FOR A CONVECTIVE DEHYDRATOR

Alexander D. Lukyanov [1], Svetlana G. Studennikova[1], Luidmila N. Alekseenko[1], David E. Bidenko[1], Vladimir Mladenović [2], Marko Petković[3], Ekaterina A. Mardasova[1]

Abstract: A microcontroller control system for a convective dehydrator has been developed. The IoT-class microcontroller provides control of the dehydrator, monitoring of the dehydration process, and transmission of telemetry data to the cloud service. Telegram bot is used as a cloud service. High-precision MEMS sensors BME-280 are used. To improve the accuracy, a mutual calibration procedure was implemented. Monitoring the air absolute moisture at the outlet of the dehydrator allows you to control the drying process in real-time. Telemetric information collected in the cloud service for the entire dehydration procedure is suitable for research and modeling of convective dehydration processes.

Keywords: convective dehydration, automatic control, microcontroller, cloud technology, MEMS sensors

Introduction

Automation is a natural process in the development of social production. To date, the issue of increasing the degree of automation in the dehydration of agricultural and pharmaceutical raw materials is relevant. The main problems in this area:

- high energy consumption during convective drying;
- lack of possibility of operational control of the dehydration process;
- duration of the process of convective drying to "constant mass", with possible overdrying

To achieve optimal temperature-time conditions of dehydration, it is necessary to be able to monitor and control dehydration parameters in real-time.

[1] Don State Technical University, Ploshchad' Gagarina 1, Rostov-on-Don, Russian Federation (alexlukjanov1998@gmail.com)
[2] University of Kragujevac, Faculty of Techical Science, Svetog Save 65, Cacak, Serbia
[3] University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia

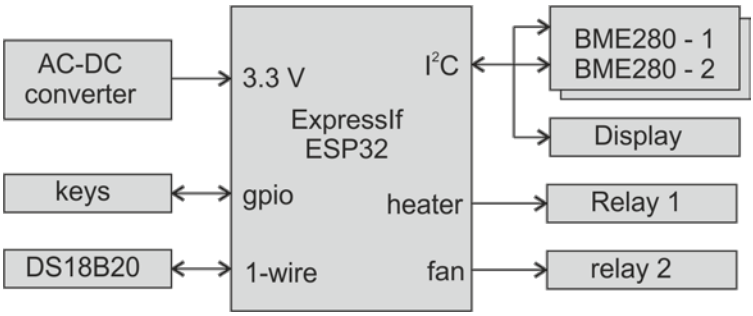
In this regard, there is a need for a device that automatically monitors and controls the dehydration process.

The aim of the work is to improve the quality of dehydrated products and reduce their cost by increasing the controllability and observability of the dehydration process and the possibility of using optimal energy-saving dehydration algorithms.

Work on the creation and improvement of control systems is carried out widely and everywhere. Modern industrial microprocessor control systems are described in (Kihara, 2020), (Dehydrator Automation System 2023). The laboratory control system is described in (Honorato, 2021). The design of control systems, conceptually close to the proposed one (but differing in implementation details) is given in (Oluwaleye, 2022), (Basista et.al., 2022).

Materials and methods

The structure of the control system is given in Figure 1. Dehydrator control system based on IoT-class SoC microcontroller ESP32 by ExpressIf Company (Espressif Systems, 2023). Such a conroller was selected due to the availability, wide set of digital interfaces, sufficient performance, low energy consumption, and the availability of two wireless interfaces: WiFi and Bluetooth.



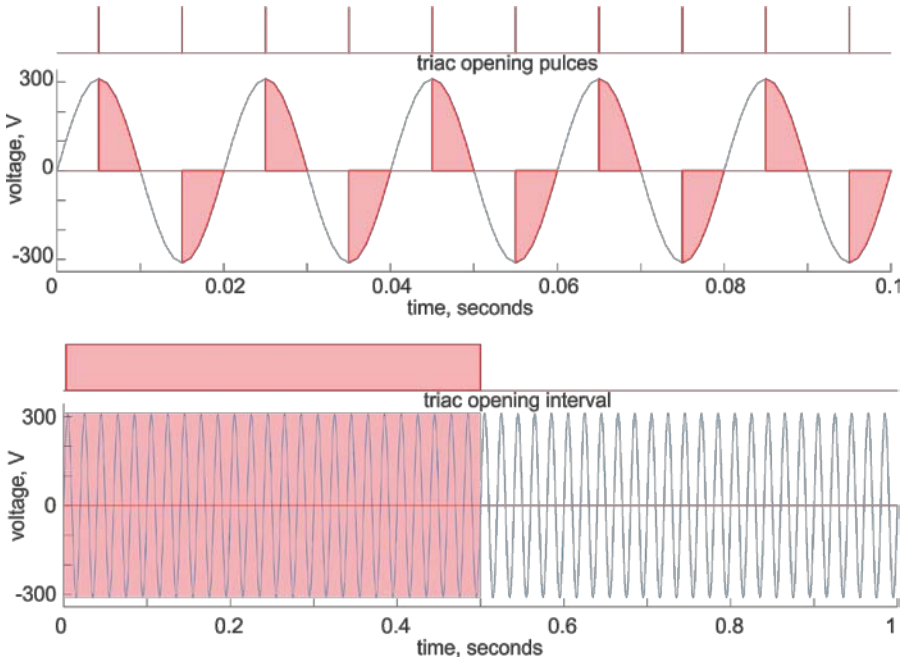
Graph 1. The structure of microcontroller-based dehydrator control system

The most significant, for understanding the features of the operation of the control system, in the structure in Graph 1 are the temperature sensor DS18B20, heater relay, and temperature, humidity, and pressure sensors BME280 (BME280, 2022). Let us dwell on their purpose in more detail.

The set "temperature sensor - heater triac relay" provides regulation and maintenance of the set air temperature at the outlet of the dehydrator heater. The

formation of the control valve on the heater relay is carried out by a digital PID - controller implemented programmatically on the microcontroller. Due to the rather large inertia of the heater, no pulse-phase control is implemented, but pseudo-PWM control (Graph 2), that is, switching on the relay for a given period with a period of 1 second. During the time the relay is turned on, a part of 100 half-period of alternating current oscillations passes to the heater, and thus the temperature is adjusted with a resolution of 1:100. Such a solution does not require synchronization of the regulator operation with the network oscillation frequency, which simplifies the circuit.

For more precise tuning of the PID controller, a mathematically developed and identified model of the heater was used, and the procedure for selecting the PID controller coefficients was carried out.



Graph 2. Pseudo-PWM principle in dehydrator heater control (bottom) compared to pulse-phase control (top)

The second feature of the presented control system is the differential calculation of absolute air humidity. For this, two BME280 MEMS sensors are used, one of which measures the air parameters at the inlet to the dehydrator, and the second at the outlet. According to the formula for saturated steam pressure versus temperature

(Buck research instruments, 2012) (Eq. 1) and the formula for absolute moisture content (Eq. 2), the value of absolute moisture content for the inlet and outlet flows is calculated.

$$p_s(\vartheta) = 6.1121 \cdot e^{\left(18.678 - \frac{\vartheta}{234.5}\right)\left(\frac{\vartheta}{257.14+\vartheta}\right)} \tag{1}$$

$$W(\vartheta, h) = 622 \cdot \frac{h \cdot p_s}{p_{atm} - h \cdot p_s} \tag{2}$$

where ϑ - air temperature, p_s - saturated steam pressure, h - relative humidity. Then ΔW - the difference between the absolute moisture content of the output W_{out} and input W_{in} air is calculated, and this value is used as a characteristic of the intensity of the dehydration process (Eq. 3):

$$\Delta W = W_{out} - W_{in} \tag{3}$$

Processes with two characteristic periods are implemented in the control system. A period of one second is used to control the heater: reading the temperature sensor, generating a control voltage, and transmitting it to the heater relay. A period of thirty seconds is used to interrogate air parameters sensors and transmit telemetric information about the dehydration process.

Telemetric information about the devoicing process and, accordingly, the dehydrator control commands are transmitted via two channels: via a serial port via a USB drive to a connected computer; via a wireless WiFi interface in the Telegram bot. Channels of reception and transmission of information are equal and can be used both simultaneously and separately. The transfer of information is carried out in text form in the ANSI encoding. An example of a line with information is given in Table 1.

Table 1. Format of the telemetry information transmission string

name	time	T_d	T_1	H_1	P_1	T_2	H_2	P_2	W	Rate
value	600	52.88	31.19	30.00	101000	26.16	38.83	100900	2.43	1000

It is also possible to download the entire package of telemetry information accumulated during the experiment from the Telegram bot application. It is most

convenient to upload information in the "machine-readable json" format. The format of one record is presented in Table 2. The order of the information fields in the last line corresponds to the field names in Table 1.

Table 2. Message format with telemetric information when uploading from a Telegram bot

name	value
"id"	366610,
"type"	"message",
"date"	"2022-02-14T23:00:03",
"from"	"DehydratorBot",
"from_id"	"user172xxxxxxx",
"text"	"85 24.06 24.09 33.35 102376.11 24.43 27.26 102417.01 24.05 1.56 0.00 0.00"

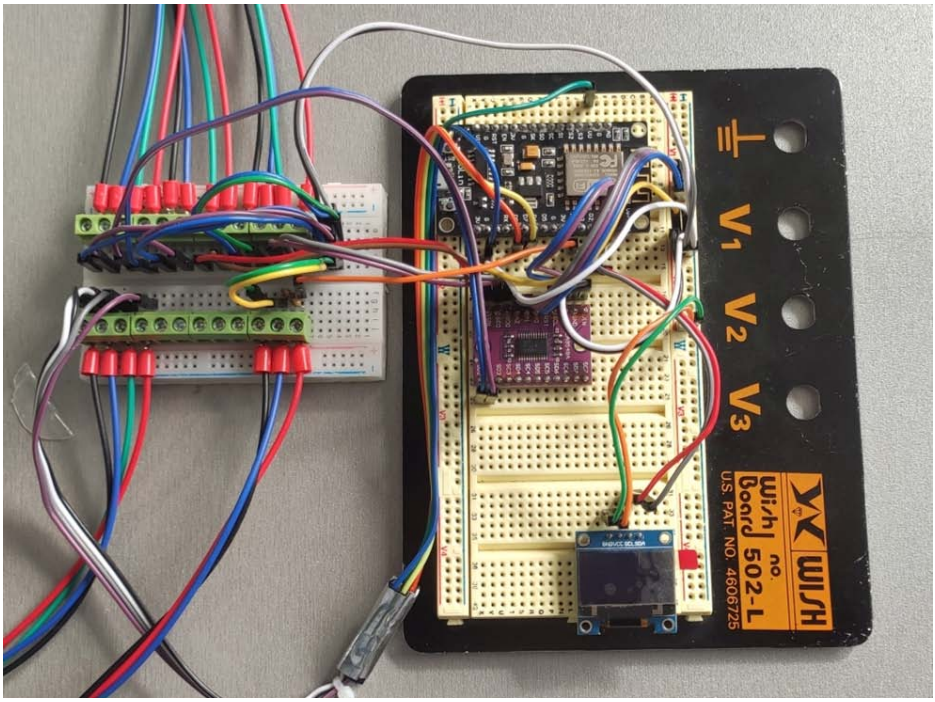
The BME280 sensor is a high-precision integrated MEMS temperature, humidity, and pressure sensor:

- humidity sensor with an error of no more than 3 % relative humidity;
- temperature sensor with an error of no more than 0.5 ℃
- pressure sensor with an error of no more than 0.25 %.

Each sensor is individually calibrated after manufacture, and the calibration values are written into registers. Nevertheless, to improve the accuracy of measuring air parameters, and considering that the difference in absolute moisture content between the inlet and outlet air is of greatest interest, a procedure for mutual calibration of the BME-280 sensors has been implemented. Calibration can be initiated after turning on the dehydrator and takes about 20-25 minutes. The duration is because, after the start of calibration, it is necessary to wait for the establishment of temperature and humidity equilibrium between the calibrated sensors (which takes about 12-15 minutes) and then record 10 readings to calculate the average differential error of the sensors and write it to the memory of the microcontroller. In the future, the values are used to correct the readings read from the sensors.

Results and discussion

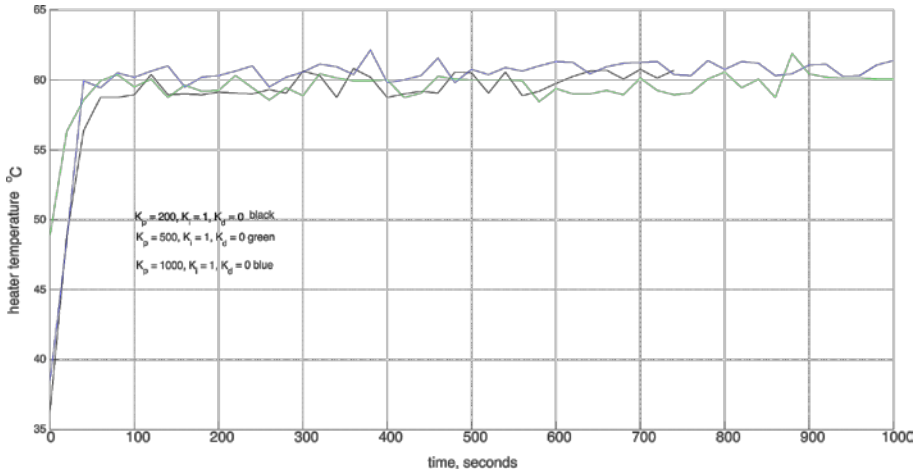
The implementation of the developed control system in the form of a layout (Graph 3) made it possible to significantly improve and expand the possibilities for conducting experimental studies on the dehydration of food products by the method of convective dehydration. Let's dwell on the achieved results in more detail.



Graph 3. The appearance of the layout of the control system

The differential calibration of the BME280 sensors made it possible to reduce the systematic error in determining the absolute moisture content from 0.7 to 0.09 mg kg^{-1} of air, which seems to be acceptable for assessing the quality of drying products.

Setting the PID controller made it possible to maintain the air temperature at the outlet of the heater with an error of no more than 0.5 °C without overshooting. Comparative graphs of temperature curves are shown in fig. 4. After tuning, the values $K_p = 1000$, $K_i = 1$, and $K_d = 0$ were chosen, which provided the entry time into the 5% control tube, equal to 40 seconds.



Graph 4. Heater PID Tuning Results

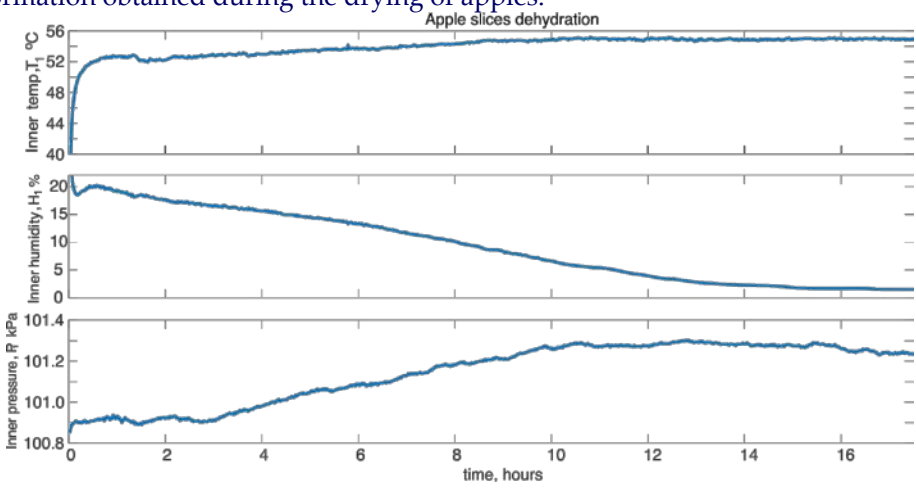
Thus, the control system provides a stable value of the set air temperature at the outlet of the heater, which will allow obtaining valid data on the intensity of the product dehydration process. At the same time, the authors have repeatedly encountered the fact that household and commercial dehydrators are equipped with relay temperature control systems operating on the interval principle, which leads to fluctuations in the air temperature after the heater, sometimes within significant limits (up to 5–10 °C).

The use of precision sensors BME280 made it possible to measure the parameters of the dehydration process with high accuracy and to calculate several derived quantities, first of all, such an invariant as the absolute moisture content of the air.

The system was tested by dehydrating apple slices in a modified household dehydrator (Graph 5).

Figure 5. A modified household dehydrator (left) and a tray of dehydrated apple slices (right)

The following graphs (Graph 6) show some channels of telemetric information obtained during the drying of apples.

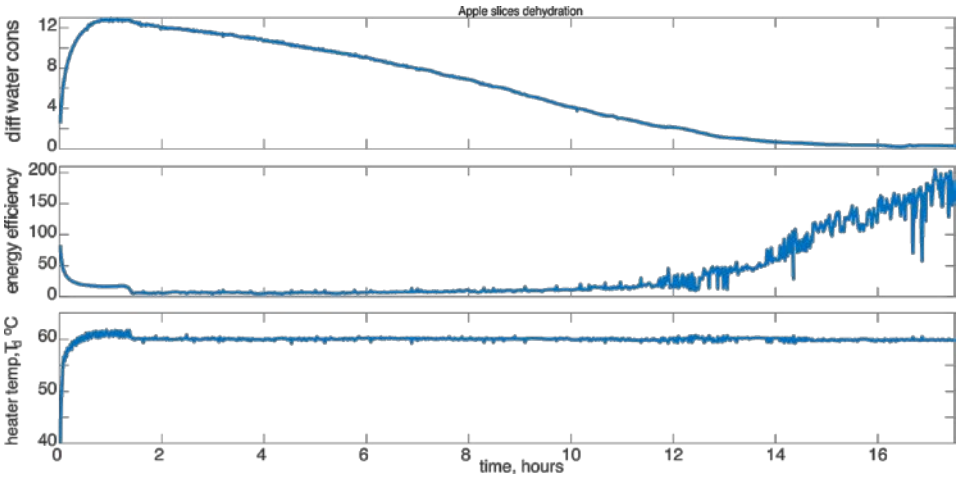


Graph 6. Graphs of telemetry information from the first (internal) BME280 sensor. From top to bottom: temperature, relative humidity, pressure

Fig. 6 shows graphs of temperature T1, relative humidity H1, and pressure P1 recorded during a complete dehydration cycle of apple slices. The duration of the process is 17 hours, and the air temperature of the heater is 60 °C. The graph of

temperature and humidity shows that as the product dries out (decrease in the relative humidity of the air at the outlet of the dehydrator), the temperature of this air increases. In general, this means that a smaller proportion of the energy brought by the air from the heater is spent on the evaporation of moisture from the product. The pressure graph shows that even during one drying cycle, the pressure can change quite noticeably for precision measurements (0.5 %), which is desirable to take into account when calculating the absolute moisture content.

However, more informative, for the analysis of processes during drying, are graphs of derived values (Graph 7; diff water – mg_{H20} kg^{-1} of air, energy costs for drying – W of power to remove 1 mg_{H20} kg^{-1} of air, and as a reference value – the air temperature at the outlet of the heater).



Graph 7. Graphs of absolute moisture content

Graph 4 allows us to formulate criteria for controlling the dehydration process. For example, it is possible to stop the drying process upon reaching a certain value of the moisture content in the air stream. Or by the loss of a certain amount of water by the product. Or by exceeding a certain threshold by energy costs, etc. Also, the collected telemetric information provides rich material for the development of mathematical models for drying products, for ex: (Filipović, 2021). But the issue of modeling is beyond the scope of this article.

Currently, the second exemplar of the control system is being assembled and adjusted for use in the Food laboratory of the Faculty of Agronomy in Cacak, University of Kragujevac.

Conclusion

The presented system of microcontroller control of the dehydrator makes it possible to control and monitor the process of dehydration of food products in a convective dehydrator in automatic mode, with the output of telemetric information to a local computer or a cloud service. The measurement time is not limited. The high accuracy of the measurements made it possible to successfully calculate such an invariant as the absolute moisture content of the air. The received telemetric information can serve both for offline analysis to study and model dehydration processes and for the operational management of the dehydration process.

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SYNTHESIS AND CHARACTERIZATION OF CROSSLINKED SILICONE NANOCOMPOSITES AND THEIR POTENTIAL APPLICATION IN FOOD INDUSTRY

Darko Manjenčić[1], Vladan Mićić[2], Anja Manjenčić[3]

Abstract: Food-grade siloxanes are inert and therefore do not emit dangerous substances. Due to these properties, it is completely harmless, unlike technical silicone. FTIR spectroscopy was used to confirm the assumed mechanism of the crosslinking reaction of siloxane. The addition of highly active silicon (IV) oxide leads to an increase in hardness and tensile strength and affects the final properties of the cross-linked materials. The analysis of the thermal properties of the synthesized siloxane materials and their nanocomposites with the addition of hydrophilic and hydrophobic particles of silicon (IV) oxide was performed using a DSC device, which showed that the increase in the proportion of nanofillers significantly affects the values of the melting temperatures of silicone elastomer materials.

Keywords: elastomers, nanocomposites, network, polymer siloxanes, thermal properties.

Introduction

Mechanical properties of elastomers are a result of the three-dimensional network structure that includes long and flexible polymer chains bonded together (Treloar, 2005), (Mark and Erman, 2007), (Owen, 2005). These chains can be connected by physical (secondary interactions) or chemical bonds (chemical bonds established between polymer chains in the crosslinking reaction). Silicone is the commercial term used for polymers and elastomers with silicone-oxygen major chains, properly called siloxanes (Hardman and Torkelson, 1991). The addition reaction of silicon hydrides (Si-H) across multiple bonds (*e.g*. vinyl groups) with using a Pt catalyst, is called catalytic

[1]College of Vocational Studies for Management and Business Communications, Belgrade,. Ratarski put 8a, 11000 Belgrade, Serbia, European University Brčko, Bijeljinska 72-74, Brčko 76100, BiH, (manjend@gmail.com);

[2] University of East Sarajevo, Faculty of Technology, Karakaj 34a, 75400 Zvornik, Republic of Srpska, Bosnia and Herzegovina

[3]University of Belgrade, Faculty of Medicine, Dr Subotića 8, Belgrade, Serbia

hydrosilation (hydrosilylation), and is one of the most known reaction in silicone chemistry (Marciniec, 2008), (Bulgakova et al., 2006). After crosslinking, a final polymer network was obtained with the presumed structure shown in Figure 1.

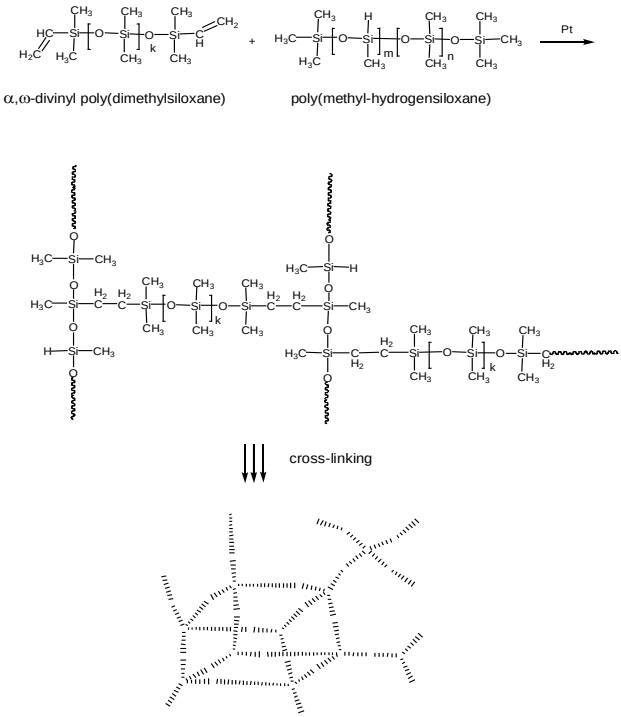

Figure 1. Structure of the obtained polymer network

In this work, influence of the siloxane matrix structure and addition of nanofillers on mechanical and thermal properties of siloxane elastomers was studied. For this purpose, polymer networks with α,ω-divinyl poly(dimethylsiloxane) and poly(methyl-hydrogensiloxane) precursors were synthesized. Hydrophobic and hydrophilic nanosilicon(IV)–oxide particles were used as fillers for nanocomposite preparation.

Materials and methods

Precursors for the siloxane elastomer were α,ω-divinyl poly(dimethylsiloxane) (Sil Vi) and poly(methyl-hydrogensiloxane) (Sil H) (ratio Sil H/Vi 50/50). Platinum complex in poly(dimethylsiloxane) containing

vinyl groups was used as a catalyst, while methyl siloxane resin containing 20% active ingredient, was used to prevent early crosslinking. Hydrophobic silicon(IV)-oxide of a specific surface area of 130 m^2 g^{-1} and an average particle size of 16 nm and hydrophilic silicon(IV)-oxide of a specific surface area of 200 m^2 g^{-1} and an average particle size of 12 nm were used as a filler in concentration of 1, 5,10 and 20 wt% . Chemical structure of prepared materials was analyzed by Fourier-transform infrared spectroscopy (FTIR) by using FTIR spectrophotometer Thermo-Nicolet Nexus 670. Mechanical properties, such as tensile strength and elongation at break, were determined according to the ASTM 412-98a standard, using a universal testing machine Shimadzu EZ-LX model. Thermal properties of siloxane elastomers were analyzed by differential scanning calorimetry (DSC) by using a DSC Q20 device.

Results and discussion

Results of the FTIR analysis of the molecular structure of synthesized elastomers are shown in Figure 2. Two sharp bands at 2961 cm^{-1} and 2905 cm^{-1} originated from the asymmetric and symmetric C-H stretching vibrations of Si-CH_3. Characteristic peaks of siloxane matrix that originated from the functional groups Si-CH_3, Si-O-Si, and Si-C are clearly observed. The absence of hydroxyl bond vibrations between 3200 cm^{-1} and 4000 cm^{-1} signifies that the surface OH groups of the fumed silica were successfully consumed by bonding with the silicon atom in the siloxane moiety in the PDMS. The absence of peaks at wavelengths greater than 3000 cm^{-1} shows the quantitative reaction of the C=C double bond in vinyl siloxanes, which confirms that the selected ratio of the reaction components, 50:50, is optimal. It can be also seen in Figure 3 that nanofillers did not chemically interact with the polymer matrix, but there were physical interactions, which resulted from the structural similarity of the filler and the matrix favoring also strong interactions among the SiO_2 hydrophilic fillers (*i.e.* SiO_2 particle agglomeration). A peak from Si-O band shifted at 1100 cm^{-1} in composites containing hydrophilic silicon(IV)-oxide, which shows weaker physical interactions of the filler and the matrix.

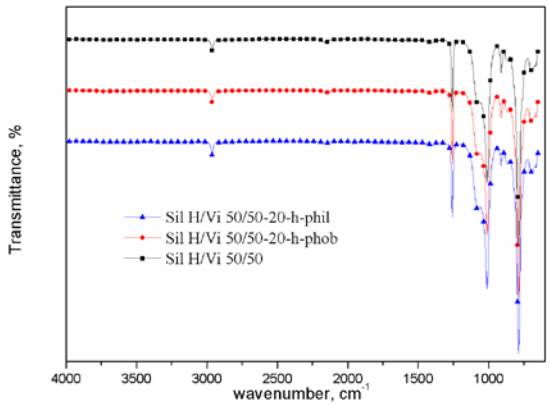

Figure 2. FTIR spectra of siloxane elastomers: hydrogen/vinyl siloxane elastomers nanocomposites (ratio Sil H/Vi 50/50) with 20 wt% hydrophilic and hydrophobic fillers

Table 1. Mechanical properties of prepared siloxane elastomers and their nanocomposites

	Tensile strength, MPa	Elongation at break, %	Shore A Hardness
Sil H/Vi 50/50	1.5	26	50
Sil H/Vi 50/50-1-h-phob	1.56	32	50
Sil H/Vi 50/50-5-h-phob	7.36	46	70
Sil H/Vi 50/50-10-h-phob	8.25	58	72
Sil H/Vi 50/50-20-h-phob	9.05	53	73
Sil H/Vi 50/50-1-h-phil	0.56	16.76	50
Sil H/Vi 50/50-5-h-phil	2.98	50.12	68
Sil H/Vi 50/50-10-h-phil	5.30	81.5	69
Sil H/Vi 50/50-20-h-phil	6.83	85.4	72

It can be concluded that the addition of nanosilicon(IV)-oxide leads to the increase in tensile strengths of the samples. The elongation at break also increases with the addition of the filler. For samples with hydrophilic fillers, a larger increase in the elongation at break was noticed, which can be explained by the lower degree of interactions of the hydrophobic siloxane matrix with a hydrophilic filler, compared to those with the hydrophobic one, resulting in a more elastic structure of siloxane nanocomposites. Stronger interactions of the hydrophobic filler with the siloxane matrix, on the contrary, results in stronger

materials with higher values of tensile strength. Addition of the filler increases hardness of composites, as it was expected, while it was not affected by the type of filler modification.

The increase in the nanofiller loading significantly affected thermal properties of siloxane elastomers. In pure silicon elastomers (ratio Sil H/Vi 50/50) melting temperatures (T_m) were about -56 °C, but with addition of the nanofiller, T_m values changed. Namely, addition of the hydrophobic silica filler resulted in an increase of T_m values, probably due to strong filler/polymer interaction. Lower compatibility of the hydrophobic matrix and hydrophilic filler caused a decrease in T_m values, because the filler/polymer interactions were weaker. With addition of both nanofiller types a slight decrease in the melting enthalpy was observed for all samples, probably because the fillers caused disordering of polysiloxane chains (Fig. 3).

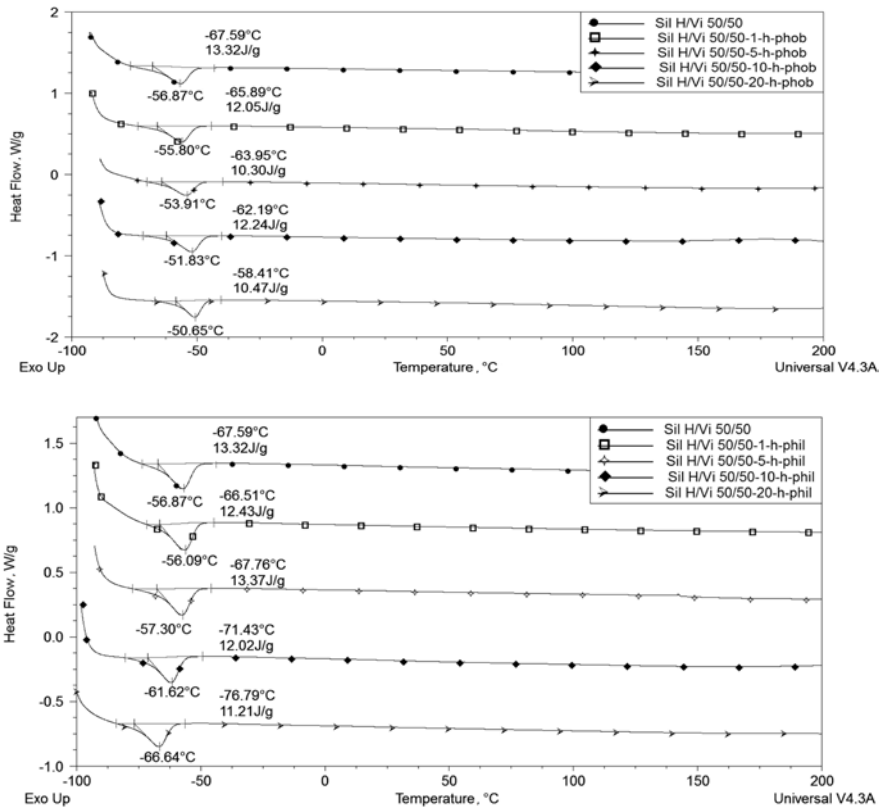

Figure 3. DSC thermograms of the silicon elastomers and their nanocomposites with hydrophobic and hydrophilic fillers

Conclusion

Siloxanes, that is, polysiloxanes, are certainly one of the most interesting class of polymers. In this paper, the influence of nanoparticles on the properties of elastomeric materials based on different siloxane precursors was investigated. FTIR spectroscopy confirmed the presumed structure of the polysiloxane and the obtained siloxane elastomeric nanocomposites. It was found that the addition of silicon(IV)-oxide nanoparticles leads to an increase in hardness and tensile strength of the siloxane elastomers. This increase is more significant at larger filler contents, that is for samples with 10 and 20 wt% of the filler. The fillers, along with inducing the increase in the strength, increased elasticity of the siloxane materials. The improvement in mechanical properties is the result of an increase in the crosslinking network density. Thus, it has been shown that the silicon(IV)-oxide fillers are suitable for adjusting the properties of siloxane elastomers. DSC results indicate that addition of the hydrophobic silica filler increased T_m values, but the hydrophilic filler caused a decrease in these values, due to the lower compatibility of the filler and the polymer matrix in the latter case.

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NUTRITIONAL VALUE AND MICROBIOLOGICAL QUALITY OF VARIOUS TYPES OF BREAD

Dobrila Ranđelović[1], Svetlana Bogdanović[1], Ivana Zlatković[1]

Abstract: For centuries, bread has been the world population's basic alimentary item. It is a bakery product made from the dough that has undergone the process of kneading, shaping, fermentation and baking. The purpose of this work is to determine the nutritional values and the microbiological quality of bread made from different types of flour. Energy value of bread was calculated based on the content of carbohydrates, proteins and fats. Microbiological quality control included bacteria that cause food spoilage, namely *Salmonella* spp., *Listeria monocytogenes, Enteriobacteriaceae, Bacillus cereus,* as well as molds and yeasts. The results of examining the energy value parameters, which are presented in our work, are in accordance with the Regulations concerning methods of physical and chemical analysis for testing quality control of grains, mill and bakery products, pasta and quick-frozen dough products. All tested microbiological parameters of white and wholemeal bread, as well as buckwheat and rye flour bread, were within the acceptable values, while corn flour bread had a slightly higher content of yeasts and molds than the prescribed standard.

Keywords: bread, nutritional value, microbiological control

Introduction

Cereal products, primarily bread, have been the world population's alimentary staples for centuries. Bread has been produced in various forms for more than 4,000 years and is considered one of the oldest foods in the world. The advice of nutritionists is that cereals should make up most of our diet (Novaković and Milosavljev, 2002). Bread is a bakery product made by mixing, molding, fermentation and baking the dough obtained by mixing milling products and water, with or without baker's yeast or other raising ingredients, kitchen salt and other raw materials, as well as from bakery product mixtures and water. According to the type of ingredients used, there are various breads:

[1]Toplica Academy of Applied Studies, Ćirila i Metodija 1, 18400 Prokuplje dobrilarandjelovic74@gmail.com

wheat bread; bread made from other types of grain; mixed bread and special types of bread (Regulations concerning the quality of grain, milled and bakery products and pasta, "Official Gazette of RS", no. 68/2016 and 56/2018). It is an important source of carbohydrates, proteins, vitamins, micronutrients and antioxidants (Rubel et al., 2015). The nutritional value primarily depends on the content of nutrients in the flour. The share of starch in certain types of white bread ranges around 57.1%. Breads in general are characterized by a slightly lower utilization of the protein component, with a lower proportion of essential amino acids such as lysine, threonine, valine and methionine. The content of mineral substances in bread decreases with the degree of milling of the flour used in the production, which means that its values are lowest in white bread. Its ratio of calcium and phosphorus content is unfavorable (Isserliyska et al., 2001). Wholemeal bread contains about 6 times more magnesium, 3 times more iron, manganese, copper and potassium and twice more calcium compared to white bread.. According to many authors, the whole grains of wheat and products made from this kind of grain contain phytic compounds, polyphenolic compounds, fibers and phosphorus that reduce the utilization of mineral substances from food (Hurell, 2003; Gargari et al., 2007). White bread also has a low vitamin content, it contains vitamins B1, B2 and PP. Due to the specified shortcomings, bread is not a complete food (Auerman, 1988).

The basic raw materials in the production of bread are flour, yeast, sugar, fats and substances that improve the organoleptic properties of the product (Lekić, 2010). Due to its nutrients, bread represents an environment suitable for the development of microorganisms, during the production and the storage period, as well as during transportation and distribution to consumers (Đukić et al., 2017). Bread is subject to easy physical, chemical and microbiological spoilage, which leads to changes in sensory properties and shortening of shelf life (Smith et al., 2010).

Bread is one of the most important items in human nutrition in many parts of the world, including our country. Developed industrial countries with a high standard of living generally have a lower consumption of bread, while poorer countries are characterized by a high average consumption of bread per capita. The consumption of bread per inhabitant in Serbia has been in constant decline in recent years. According to official statistics, the annual average consumption per household member in 2006 was 101.5 kilograms of bread and pastries, while in 2021 it was 60.5 kilograms (Population Consumption Survey, 2021).

The aim of this work is to determine the nutritional values and microbiological quality of bread made from different types of flour.

Materials and methods

In this work, five different, commercially available types of bread were examined, namely white bread (sample 1), wholemeal bread (sample 2), buckwheat bread (sample 3), rye bread (sample 4) and corn flour bread (sample 5). W heat bread contains at least 90% of wheat flour. Wholewheat bread is made from wheat flour or wholewheat bran. Buckwheat bread is made from at least 30% buckwheat flour, calculated from the total amount of flour used. Rye bread is produced from at least 70% of different types of rye flour, calculated from the total amount of flour used. Corn bread is produced from at least 60% corn flour or other corn mill products (Regulation concerning the quality of grain, mill and bakery products and pasta, 2018).

The energy value of bread was calculated based on the content of carbohydrates, proteins and fats. All methods were performed based on the Regulations concerning methods of physical and chemical analysis for testing quality control of grains, mill and bakery products, pasta and quick-frozen dough products (Official Gazette of SFRY 74/88).

Microbiological quality control included bacteria that cause food spoilage, namely *Salmonella spp., Listeria monocytogenes, Enteriobacteriaceae, Bacillus cereus,* as well as molds and yeasts. All the methods were based on the SRPS EN ISO standard, namely *Salmonella* spp. SRPS EN ISO 6579-1:2017, *Listeria monocytogenes* SRPS EN ISO 11290-1:2017, E*nterobacteriaceae* SRPS EN ISO 21528-2:2017, *Bacilus cereus* SRPS EN ISO 7932:2017 and SRPS EN ISO 21527-2:.2008 for molds and yeasts. During the microbiological quality control, one bread was taken at random and divided into 5 parts, each of them was examined separately, and all five parts made up one sample.

Results and discussion

The obtained values of carbohydrates, proteins and fats are presented in Table 1. The values of protein and fat content are given in g/100g. All the results of the examined parameters in our work are in accordance with the Regulations concerning methods of physical and chemical analysis for testing quality control of grains, mill and bakery products, pasta and quick-frozen dough products.

Table 1 Results of chemical analysis of bread

Sample number	Carbo-hydrates (g/100 g)	Proteins (g/100 g)	Fats (g/100 g)
Sample 1	48,1	9,0	3,2
Sample 2	55,6	13,1	4,3
Sample 3	66,5	12,2	3,3
Sample 4	46,4	9,4	0,4
Sample 5	31,8	5,5	8,1

All the tested microbiological parameters for white and integral bread, as well as for bread made from buckwheat and rye flour, were within the acceptable values. Cornmeal bread had a slightly higher content of yeast**s** and molds than the prescribed standard (1.03×10^2 cfu/g). Similar results were obtained by Eglezos et al. (2010) who explain the obtained results by using raw materials that are more susceptible to microbiological spoilage.

Table 2. Microbiological parameters of quality control of breads made from different types of flour

Microorganisms	Limit values (cfu/g)		Sample determined value Sample 1	Sample determined value Sample 2	Sample determined value Sample 3	Sample determined value Sample 4	Sample determined value Sample 5
	m	M					
Bacillus cereus	10^2	10^3	No presence	No presence	No presence	No presence	No presence
Enterobacteriaceae	10	10^2	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g	<10 cfu/g	< 10 cfu/g
Yeasts and molds	10^2	10^3	< 10^2 cfu/g	<10^2 cfu/g	< 10^2 cfu/g	< 10^2 cfu/g	>10^2 cfu/g
Salmonella spp.	must not be present in 25g		No presence	No presence	No presence	No presence	No presence
Listeria monocytogenes	must not be present in 25g		No presence	No presence	No presence	No presence	No presence

n - number of units that make up the sample ; c – number of sample units that show values between m and M

The absence of indicator organisms does not fully guarantee that the controlled food does not contain pathogenic microorganisms whose presence is not routinely controlled (Trajković Pavlović et al., 2010). For this reason, it is recommended that those employed in the food industry, that have the greatest responsibility for the safety of their products, develop and implement their own procedures and instructions for ensuring the application of the principles of good production practice and good hygiene practice, which also implies the application of elements of the HACCP system (Trajković Pavlović, 2014).

Conclusion

Bread is a mass-consumed product accepted by the consumers as healthy and safe. Based on the results of testing the energy value of bread, as well as the microbiological quality control, we can conclude that bread made for commercial use meets the criteria of the Regulations, and that it is completely safe for use.

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EFFECT OF PACKAGING ON HEALTH SAFETY OF SAUSAGE

*Senita Isaković [*2], Enver Karahmet [*1], Saud Hamidović [1], Jasmina Tahmaz [1] and Ajla Smajlović[2]*

Abstract: The main goal of this study was to determine the impact of the type of packaging to health safety sausage, and therefore the microbiological compliance with the requirements of the Regulation on microbiological criteria for foodstuffs. The study included the determination of the presence of bacteria of the genus: *Salmonella, E.coli, Enterobacteria, Campylobacter, Listeria monocytogenes, and sulfitereducing Clostridiae* as well as the total number of bacteria within each individual product. According to estimate the effect of the type of packaging (vacuum, MAP and bulk) to the safety of sausages was performed collecting samples of sausages packed in a vacuum packaging, modified atmosphere packaging of bulk and different manufacturers for each type of packaging.

As a result of the study, out of 10 analyzed samples, bacteria from the *Enterobacteriaceae* family in the range of values from 1.8 to 2.55 CFU/mL., *E. coli*, in the range of 0, were found by isolation on selective mediafrom 0.51-1.23 CFU/mL. Total bacteria ranged from 2.6-4.02 CFU/mL. Yeasts and molds in the samples tested were between 2.22 - 3.84 CFU/mL. Microbiological tests did not reveal any bacteria from the following groups: *Salmonella* spp., *Listeria* and sulphite-reducing *Clostridia*. It was found that all samples correspond to the Regulation on microbiological safety.

Keywords: packaging, sausages, safety, microbiology, bacteria

Introduction

Sausages are products of different types and amounts of minced meat, mechanically deboned meat, fatty tissue, connective tissue, offal and additives, which are stuffed into natural or artificial casings or shaped without casing. The main ingredients used in the sausages production are the skeletal muscles of slaughtered cattle, calves, pigs, sheep, poultry and other species. However,

[1] University of Sarajevo, Agriculture and Food Sciences Faculty, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina, enverkarahmet@yahoo.com

[2] Pharmacy & Bio" d.o.o. Šefika Loje 22, 71000 Sarajevo, Bosnia and Herzegovina, senita.ciklas@hotmail.com

different skeletal muscles differ not only in their fat, water, and protein content, but also in their water binding, color, emulsifying properties, etc. The control of moisture, fat and protein is difficult due to the fact that it is not possible to obtain a high degree of uniformity of meat and fat parts in different sausage formulations. Therefore, in order to achieve a precisely formulated product, the raw material must be classified according to fat, moisture, proteins, as well as the type of animal from which it originates (Operta, 2012).

Both meat and meat products are very sensitive to the action of external factors, such as light, oxygen, moisture and microorganisms. Properly selected packaging and applied packaging conditions provide protection from these influences, as well as preservation of nutritional properties for the declared shelf life. Consumers are increasingly interested in naturally preserved and high-quality food products that have not been chemically treated. This trend imposes on manufacturers the necessity of applying modern packaging procedures that ensure the extension of the product's sustainability, while reducing the need for the use of artificial additives and preservatives. Fresh meat and meat products exposed to ambient air provide an excellent substrate for the development of most bacteria.Spoilage begins immediately, and if other factors favoring their growth are present, e.g. high temperature, it is almost certain that the product will be unusable after a few hours. Ordinary vacuuming can extend the shelf life of meat, but it dries out the food, and if pieces of meat are packed, they stick to each other. There are no such problems with MAP. Carbon dioxide is of particular interest when packing meat and meat products, as an inevitable protective gas. It is very effective against the most common microorganism that attacks meat - Pseudomonas. Oxygen gives fresh meat a beautiful, red color. In dried meat products, the presence of oxygen is harmful and up to 0.5% in MAP is allowed, in order to avoid discoloration and rancidity of the fat. Nitrogen is particularly suitable for thermally processed meats, and meat products *(*Freceet al., 2012*).*

A large number of food products, including meat products such as sausages, need to be protected from the influence of oxygen during storage, which can be done in three ways: by eliminating air from the packaging unit, by replacing residual air in the packaging with an inert gas (most often nitrogen) or by packaging in a modified atmosphere (MAP).Food packaging (MAP - Modified Atmosphere Packaging) is a special treatment of ready-made products that protects foods containing fats and aromatic substances from oxidation, maintains food freshness, and ensures a longer shelf life of products without discoloration. Packing meat in a vacuum is an alternative to packing fresh meat,

suitable for storing products for up to three weeks.In vacuum packaging, by removing air in an oxygen-impermeable package, anaerobic/microaerophilic conditions are created. The oxygen left in the packaging turns into carbon dioxide due to the respiration of the meat tissue and bacterial activity. The created anaerobic conditions and the inhibiting effect of CO2 suppress the growth of *Pseudomonas* and *Achromobacter species* and enable the growth of facultative anaerobes such as *Lactobacillus* and *Leuconostoc* spp. (Vereš, 2004). Bulk packages are cheaper, because the price does not have to include the additional costs of individual packages. When buying bulk goods, consumers pay for the product itself, not for packaging or advertising. Many bulk products have fewer preservatives and additives than packaged foods. This is of great importance for everyone, but above all for those who suffer from allergies and who are more sensitive to these food additives. However, the problem with bulk food is that it can easily become contaminated. In addition, there is also the possibility of contamination with viruses and bacteria of human origin.Sausages may also show some errors, which are manifested by the appearance of a cavity in the middle, an over-dried edge as well as its dark color, while uneven salting causes more moisture, and thus faster spoilage. In this research, the focus is on vacuum packaging, modified atmosphere and samples of sausages in bulk packaging from five different manufacturers (Markov et al., 2013; Karahmet et al. 2021.).

Material and methods

Ten samples of sausages packed in: vacuum packaging, in a modified atmosphere and samples of sausages in bulk packaging from different manufacturers were taken as the material of this research. Permanent, semi-permanent and cured sausages were used for this research. Sampling for microbiological analysis was done by the method of random sampling by shopping in shopping centers. For bulk packaging, samples of Bosnian sudžuk from local producers were used.

When preparing the tested products, the surface layer was removed and then 50-70g of each product was cut and crushed in a grater. 20g of each chopped sample was weighed into an Erlenmeyer container with 180 mL of physiological solution. Shredded samples of meat products were homogenized in physiological solution using a rotary shaker. After that, the solutions were diluted to the final dilutions that were used for microbiological analyses.

Microbiological analyzes of the samples used were performed in the Microbiology Laboratory of the Faculty of Agriculture and Food in Sarajevo. The microbiological correctness of the samples was checked for the presence of*Salmonella, E.coli, Enterobacteriaceae, sulfite-reducing Clostridia, L. monocytogenes, Campylobacter,* and the total number of bacteria. The obtained results are to be interpreted on the basis of reference values for bacteria, the presence of which was determined according to the Rulebook on microbiological criteria for food (Official Gazette, BiH number 11/31).

Results

Comparing the obtained mean values of the presence of certain types of microorganisms in bulk packaging, it can be said that aerobic mesophilic bacteria predominated in all three Sudjuk samples (3,38-4,02CFU/mL) and total yeasts and molds (3,22-3,84CFU/mL). As mentioned before, *Salmonella, sulfite-reducing Clostridia and Listeria* were not detected, but they were found*E. coli* (1,12–1,23CFU/mL), and*Enterobacteria*(2,13–2,55CFU/mL).

Table 1 Mean values of microorganisms found in bulk packaging

PACKAGING	*Microorganisms*						
Bulk packaging	*Salmonella* (CFU/mL)	*E. coli* (CFU/mL)	*Enterobacteria*(× 10^3 CFU/mL)	Total number of m.o. (× 10^3 CFU/mL)	*Listeria (CFU/ml)*	*sulfitreducing Clostridia* (CFU/mL)	*Total yeasts and molds* (×10^2 CFU/mL)
Sudžuk „Šukurica“	n.d.	1,12	2,13	4,02	n.d.	n.d.	3,77
Sudžuk „Babić“	n.d.	1,23	2,55	3,38	n.d.	n.d.	3,84
Sudžuk „Džakovac“	n.d.	1,20	2,33	3,54	n.d.	n.d.	3,22

*n.d. – not detected

Table 2 Mean values of microorganisms found in vacuum packaging

PACKAGING	*Microorganisms*						
Vacuum	*Salmonella* (CFU/mL)	*E. coli* (CFU/mL)	*Enterobacteria* (× 10^3 CFU/mL)	Total number of m.o. (× 10^3CFU/mL)	*Listeria (CFU/ml)*	*sulfitreducing Clostridia* (CFU/mL)	*Total yeasts and molds* (× 10^2 CFU/mL)
Merak sudžuk	n.d.	0,88	1,87	3,0	n.d.	n.d.	2,7
Sudžuk Semić	n.d.	0,75	2,05	3,2	n.d.	n.d.	3,24
Čajna K plus	n.d.	0,62	2,43	3,4	n.d.	n.d.	3,64
Visočka ljuta	n.d.	0,51	2,15	2,7	n.d.	n.d.	2,66
Krajiška ljuta	n.d.	0,58	2,30	2,6	n.d.	n.d.	2,22

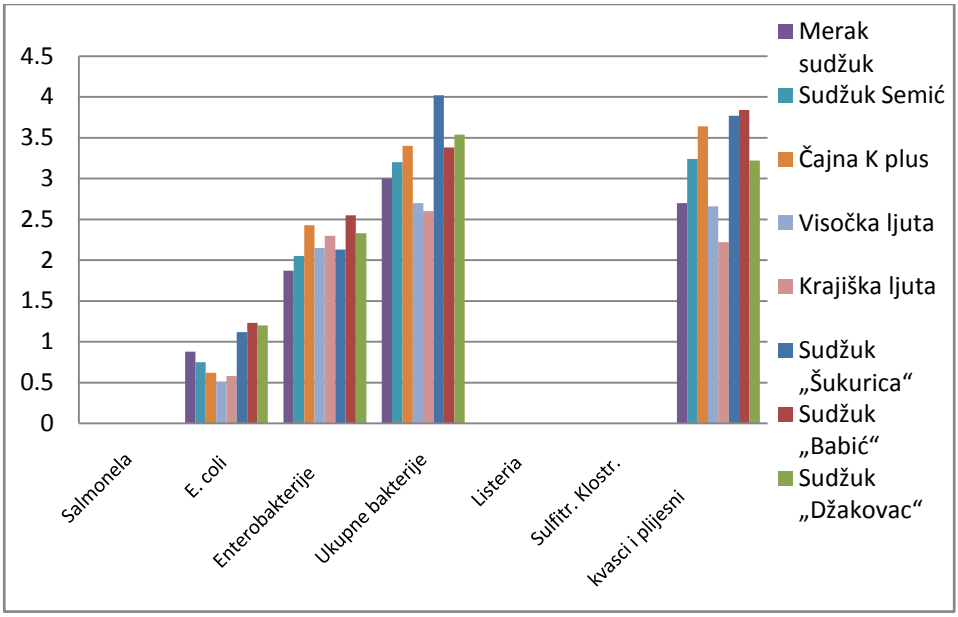
*n.d. – not detected

Table 3 Mean values of microorganisms found in MAP packaging

PACKAGING	***Microorganisms***						
MAP	*Salmonella* (CFU/mL)	*E. coli* (CFU/mL)	*Enterobacteria* (× 10^3 CFU/mL)	Total number of m.o. (× 10^3CFU/mL)	*Listeria (CFU/ml)*	*sulfitreducing Clostridia* (CFU/mL)	*Total yeasts and molds* (× 10^2 CFU/mL)
Slajsani kulen	n.d.	0,76	2,12	3,12	n.d.	n.d.	2,54
Slajsani sudžuk	n.d.	0,82	2,43	3,18	n.d.	n.d.	2,48

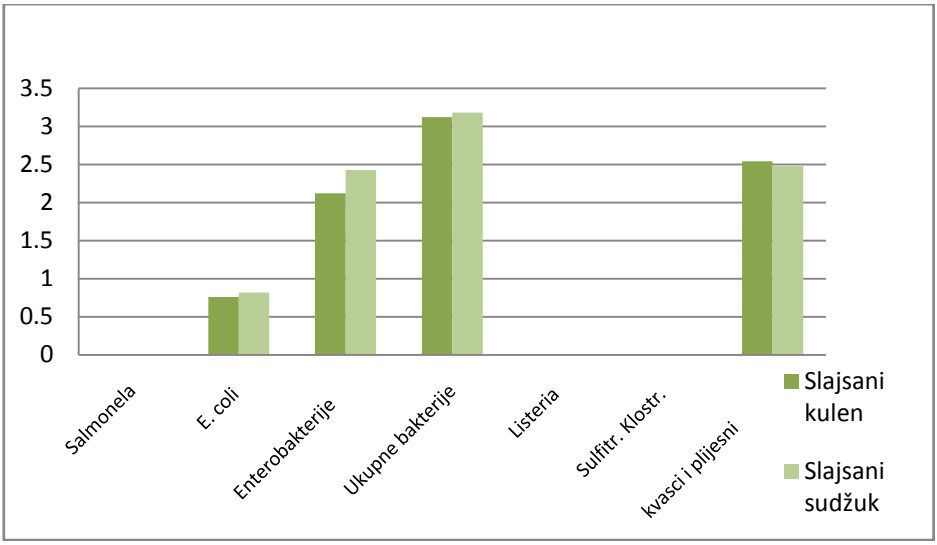
*n.d. – not detected

Analysis of the microbiological results of samples of bulk packaging of sudjuk from different manufacturers revealed that no bacteria from the group of *Salmonella* spp., *Listeria and sulfite-reducing Clostridia* were found in any sample. An approximately equal number of grown colonies (CFU) was found in all three samples*E. coli (*1,12-1,23 CFU/mL*), Enterobacteria (*2,13-2,55CFU/mL*).* The number of grown colonies of total bacteria was the highest in the sudjuk produced in the "Šukurica" meat processing plant4,02 CFU/mLwhile in the other two samples it was *(*3,38-3,54 CFU/mL*).*



Graph 1 Comparison of the ratio of bacteria found in vacuum packaging samples

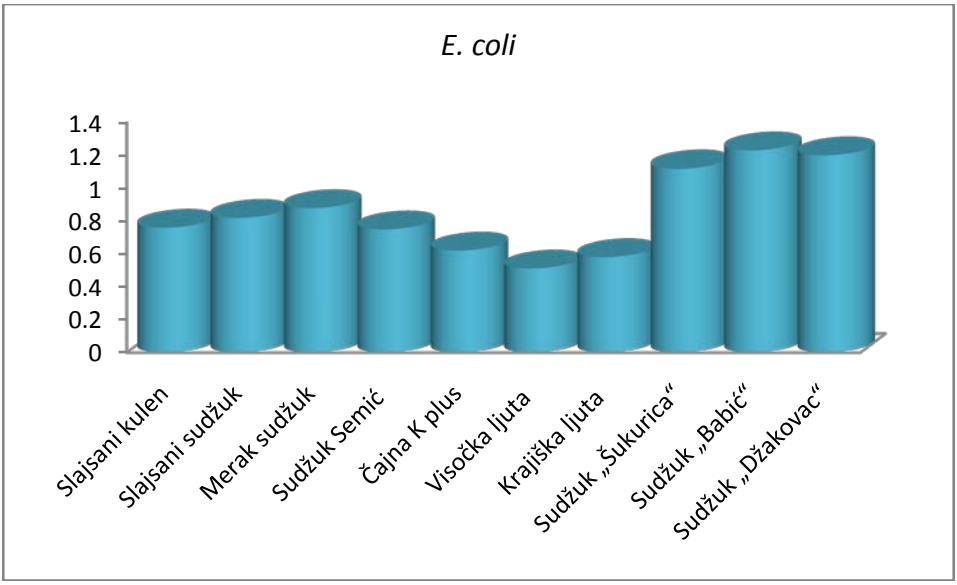
The microbiological examination of the tested samples of vacuum packaging of meat products found that no grown colonies of *Salmonella* spp., *Listeria* and sulfite-reducing *Clostridia* were found in any sample. The number of grown *E. coli* colonies was the lowest in the samples of "Visočka ljuta" *(*0,51 CFU/mL*)*and„Krajiška ljuta“ (0,58 CFU/mL)in the case of other samples, these values were slightly higher, so "Merak sudžuk" had 0,88 CFU/mL, but „Sudžuk Semić“0,75 CFU/mL.



Graph 2 Comparative display of the ratio of bacteria found in modified atmosphere packaging (MAP) samples

Salmonella spp., *Listeria monocytogenes and sulfite-reducing Clostridia* bacteria were not present in two samples of sliced sudjuk and sliced kulen. A uniform presence was observed *E. coli (*0,76 i 0,82 CFU/mL*).* The total number of micro-organisms was also equal *(*3,12 and 3,18 CFU/mL*).* The average number of grown colonies of total yeasts and molds was2,54 and 2,48 CFU/mL.

The highest average number of *E. coli* colonies grown was recorded in bulk samples in all three samples and was approximately the same (1,12, 1,20 and 1,23 CFU/mL). A significantly lower number of grown colonies was found at "Visočka ljuta" and "Krajiška ljuta" *(*0,51 and 0,58 CFU/mL*).* In other samples, this value was slightly higher, from 0.61 CFU/mL recorded in "Čajna K plus" to0.88 CFU/mL in "Merak sudžuk".



Graph 3 Comparative display of the number of E. coli found in all tested samples

The average number of grown colonies of microorganisms from the Enterobacteriaceae group was significantly higher compared to the presence of *E. coli* in the samples. Namely, the average number of grown colonies was the lowest found in "Merak sudžuk" (1.87 CFU/mL), and the highest in "Čajna K plus" (2.43 CFU/mL).

The mean values of detected yeasts and molds in the tested samples varied depending on the type of packaging, so in products packaged in MAP the value was 2.48-2.54 CFU/mL. In samples from bulk and vacuum packaging, those values were higher than 2.7-3.84 CFU/mL. Regardless of the obtained values, all products were within the requirements of the Rulebook, and are therefore considered safe for health.

Discussion

The composition of the microflora is characteristic for all types of fermented sausages, which is conditioned by the hygienic quality of the raw materials and additives used, technological procedures and microclimatic ripening conditions (Zdolec et al., 2007). Microorganisms that represent undesirable

micropopulations of fermented sausages mainly come from the used muscle and fat tissue, spices and other additives and casings.

Representatives of the *Micrococcaceae, Lactobacillaceae, Pseudomonadaceae* and *Enterobacteriaceae* families are the most common contaminants. In addition, species of the genera *Acinetobacter, Psychrobacter* and *Bacillus,* otherGram-positive rod-shaped bacteria and yeastsare represented. *Lactobacilli* in sausage stuffing cause excessive sour taste, discoloration and gas bubbles. *Lactobacilli*, as well as some species of the *Leuconostoc* genus, also cause the appearance of threads that stretch out. Putrefactive changes are caused by certain species from the families *Enterobacteriaceae, Pseudomonadaceae and Bacillaceae (*Tešanović and Kalenjuk, 2017*).*

Nielsen et al. (2008)state that the most frequently isolated *halophilic yeasts* from fermented meat products are *Debaromyces hansenii, Candidafamata, Candida zeylanoides, Trichosporon*spp., *Cryptococcus* spp. *and Rhodotorula* spp., as yeasts also play an important role in sausage ripening. Namely, yeasts contribute to the development of the sensorial properties of fermented sausages, due to their lipolytic and proteolytic activity(Alagić et al., 2008).The population of yeasts(> 5 log10CFU/g)and *coagulase negative cocci* (3.5 log10 CFU/g)did not significantly change during ripening(Tešanović and Kalenjuk, 2017).

Compared to research on industrial fast-fermented sausages, traditionally produced home-made sausages show poorer hygienic quality of raw materials and prepared filling, and a slower acidification process with consequent slower suppression of undesirable microflora. In terms of product safety, it is significant that no pathogenic bacteria were found in the filling at the end of the production process. The finished product met the microbiological conditions for fermented sausages prescribed by the Rulebook on microbiological criteria for food.

Regardless of individual deviations between products and type of packaging, there were no significant differences between individual products in the mean value of the number of detected microorganisms, and not a single product deviated from the values specified in the Regulation on microbiological criteria for food.

Conclusion

After analyzes of sausage samples on selective media, the values of confirmed and found bacteria ranged from: *Enterobacteriaceae* in the range from 1.8 to 2.55 CFU/mL, *E. coli* from 0.51-1.23 CFU/mL, average value of total

bacteria ranged from 2.6-4.02 CFU/mL in all tested samples, the value of yeasts and molds in the tested samples was between 2.22-3.84 CFU/mL.Microbiological tests did not find bacteria from the group: *Salmonella* spp., *Listeria and sulfite-reducing Clostridia*. The average values of the grown bacterial colonies were within the scope of the Rulebook on microbiological criteria for food, which again confirms that all products are suitable for human consumption from a health point of view. In the end, it can be concluded that the packaging method did not significantly affect the presence of certain groups of microorganisms.

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THE INFLUENCE OF MICRONIZATION OF CEREALS AND LEGUMES ON FEED CONVERSION, DIGESTIBILITY, AND DAILY GAIN OF WEANED PIGLETS

Danka Milovanović[1], Valentina Nikolić[1], Slađana Žilić[1], Marijana Simić[1], Beka Sarić[1], Snežana M. Jovanović[1], Marko Vasić[1]

Abstract: The main aim of this study was to assess the effect of the micronization process applied on cereals and legumes, as feed components, on the efficiency of feed conversion, digestibility, and daily gain of weaned piglets. The results showed that the digestibility of the investigated micronized maize and wheat flakes was higher than that of the respective raw grains. The digestibility of the feed mixture prepared with micronized cereals and legumes was higher (84.74%) than that of the commercial feed mixture (80.27%). The feed mixture prepared with micronized cereals and legumes manifested beneficial effects on weaned piglets' daily gain, feed conversion, and digestibility in the feeding trial.

Keywords: cereals and legumes, micronization, feed conversion, digestibility, daily gain of piglets

Introduction

Micronization, a short-time high-temperature process that utilizes electromagnetic radiation in the infrared region to rapidly heat materials, is frequently used to modify the functional properties of the biomolecules in cereal grains and legumes. The grain softens and swells before bursting when infrared rays enter the grains core and heat it up, causing the water molecules to vibrate. Critical temperatures can result in destruction and splattering, so the grain needs to be transported to the rollers and pressured in order to obtain the form of flakes (Žilić et al., 2010). Reduced fiber particle size with micronization treatment effectively destroys dense fibrous matrix formations while increasing surface area and porosity of the feed particles (Dhiman & Prabhakar, 2021).

Digestibility of the feed is one of the most important quality parameters used in the diet formulation practices for different stages of pigs (Pomar et al., 2009). Optimizing the diet based on the nutrient digestibility values of the feed ingredients is necessary to provide pigs a diet that meets their nutritional needs.

[1]Maize research Institute, Zemun Polje, Slobodana Bajića 1, Belgrade-Zemun, Serbia (dankamilovanovic10@gmail.com)

The average daily gain of pigs fed the same amount of a meal but with varied nutritional requirements can, however, vary (Verschuren et al., 2021).

The physicochemical grain properties and a variety of other factors may affect the digestibility of a feed (Gómez et al., 2016). Cereal grains and legumes are exceptionally rich in nutrients, especially dietary fiber, B vitamins, minerals, as well as phytochemicals all potentially health-beneficial. However, the fiber-rich grain pericarp is highly resistant to digestion. The main source of energy in diets for non-ruminants is starch. Compound diets made up of grains and legumes depend on the breakdown of starch as a key factor in determining the dietary energy value. Some starches are primarily digested in the small intestine, whilst others (especially those found in field peas) are linked to lower net energy values and potential digestive issues (Wiseman, 2006). Micronized grains and legumes, such as barley, maize, wheat, sorghum, lentils, and peas, have a higher degree of starch gelatinization than raw grains, which enables higher digestibility (Bellido, et al., 2006). Pigs can effectively utilize whole, micronized soybeans, making them a potentially helpful source of protein and energy (Lawrence, 1978).

The objective of this study was to investigate the effect of the micronization of feed components, i.e. cereal grains and legumes on feed conversion, *in vitro* digestibility, and daily gain of weaned piglets.

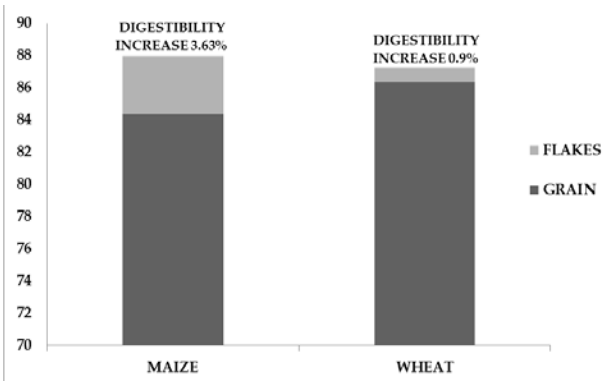
Materials and methods

The plant material used in this study comprised of: maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), barley grain (*Hordeum vulgare* L.), soybean (*Glycine max* (L.) Merr.), field peas (*Pisum sativum* L.), as well as flakes of the respective cereals and legumes. An experimental dry feed mixture for weaned piglets (up to 25 kg) prepared in the pilot plant of the Maize Research Institute, Zemun Polje was also used for the analyses. The experimental dry feed mixture from the pilot plant consisted of: ground micronized maize grain (33%), ground micronized wheat grain (10%), ground micronized barley grain (12%), field peas (10%), soy semolina (26.5%), fish meal, fodder yeast, dicalcium phosphate, fodder chalk, animal feed salt, premix, and acidifier. A commercial feed mixture for weaned piglets (up to 25 kg) obtained under producers' specification was procured from a local supplier and used as a control for the comparison of the investigated parameters. *In vitro* dry matter digestibility of the samples was performed by the pepsin-cellulase Aufréré method (2007) based on the enzymatic solubility of the feedstuff. The grain samples were subjected to the process of dry micronization (infrared treatment) at the temperature of 145 °C for 40 s in a

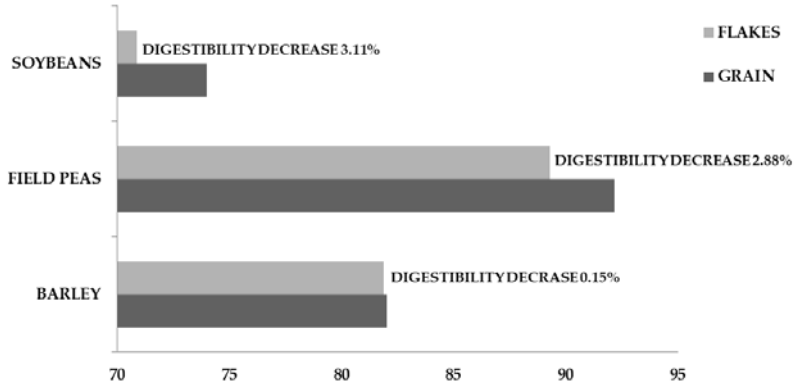
micronizer (model M1044/77, Micronizing Company UK Ltd, Woodbridge, UK). Infrared rays were applied to roast the kernels of the investigated cereals and legumes, which were then flaked under the pressure of rolls. The cereal grains, legumes and micronized flakes were ground on a a laboratory mill with mesh 0.5 mm (Perten MILL 120 CE, Perten Instruments in Hägersten, Sweden). The feeding trial was conducted on 20 weaned piglets in a local pig farm. Ten were fed with a commercial dry mixture for weaned piglests, and the remaining ten were fed a dry feeding mixture produced in the pilot plant of the Maize research Institute, Zemun Polje.

Results and discussion

The *in vitro* dry matter digestibility of the investigated untreated cereals and legumes, as well as micronized flakes obtained from these grains are shown in Graph 1 and Graph 2. The determined digestibility ranged from 81.87% in micronized barley flakes, to 92.17% in raw field peas. The micronization positively affected the digestibility of the maize and wheat grain, demonstrated by the increase of maize flakes digestibility for 3.63%, and wheat flakes 0.9% (Graph 1). However, a decreas in digestibility of soybeans and field peas after micronization and flaking was noticed (Graph 2). Previous studies reported that in comparison to starch from cereals, the total tract digestibility of legume starch was lower, and rolling, as opposed to grinding, decreased the total tract digestibility of both cereal and legume starch (Larsen et al., 2009).



Graph 1 Changes in *in vitro* digestibility of maize and wheat grain after after the micronization and flaking (% d.m.)

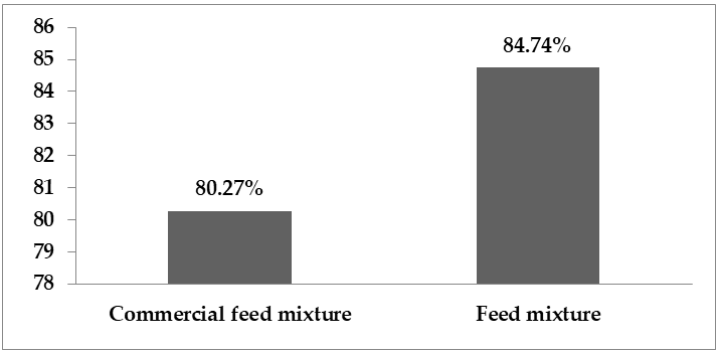


Graph 2 Changes in *in vitro* digestibility of soybean, field peas and barley grain after after the micronization and flaking (% d.m.)

Berrocoso et al. (2014) found that due enhancements in ileal mucosal morphology and nutrient digestibility the use of added value soy products such as micronized soybean meal or soy protein concentrate in diets for healthy pigs weaned at 30 days of age is advised.

Two feed mixtures were used in the feeding trials on piglets weighing up to 25kg. The difference in the digestibility of the investigated feed mixtures is shown in Graph 3. The mixture with micronized grains and legumes showed. 4.47% higher digestibility than the commercial blend.

In the feeding trial with pigs weighing up to 25 kg, the impact of nutrient digestibility was used to calculate daily gain and, consequently, food conversion. Table 1 shows the results of the feedeng trial. The findings of our study in general support the hypothesis that micronization improves the digestibility by incresing it, and hence fascilitates a better nutrient utilization.



Graph 3 Digestibility of feed mixtures (% d.m.)

Table 1 Daily weight gain and feed conversion

	Commercial mixture	Mixture of micronized grain
Daily weight gain (kg)	0.28	0.44
Feed conversion (kg)	4.06	2.15

Piglets fed the commercial combination gained 280 g daily and had a feed conversion of 4.06 kg, as opposed to 440 g for piglets fed a combination of micronized grains and a feed conversion of 2.15 kg.

Conclusion

The results obtained in our study confirmed that micronization positively affected the cereals and legumes regarding feed digestibility. The increased digestibility of the dry feed mixture prepared with micronized cereals and legumes had a beneficial impact on the performance of the weaned piglets in the conducted feeding trial. The weaned piglets fed with the dry feed mixture with micronized ingredients showed higher daily weight gain and lower feed conversion.

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ANTIOXIDANT PROPERTIES AND BIOLOGICAL ACTIVITY OF FRUIT WINES

Aleksandar Petrović[1], Ivana Plavšić-Janjatović[1], Nikolina Lisov[1], Maria Čebela[2], Uroš Čakar[3*], Ivan Stanković[3], Brižita Đorđević[3]*

Abstract: The fruit wines from blueberry were made by microvinification procedure. Wines were produced in the absence or presence of sugar and/or enzymatic preparation glycosidase (EPG). Selected phenolic acids were quantified using UPLC/MS-MS analysis. Total phenolic content (TPC) was determined by the Folin-Ciocalteu method. Also, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing ability of plasma (FRAP) methods were applied. The α-glucosidase inhibitory activity of blueberry wines was also investigated. Wines made with addition of sugar and EPG showed the best results. Phenolic profile and biological activity of blueberry wine depended from microvinification procedure which was used in the production.

Keywords: blueberry wine, phenolic compound, antioxidant activity, anti radical activity, α-glucosidase inhibitory activity

Introduction

Fruits and vegetables are essence part of balanced diet and their regular consumption (400 g every day), as fresh or processed may positively affect human health (Joshipura et al., 2001). Antioxidant compounds are responsible for health promoting effects of food, since they prevent development of chronic non-communicable diseases such as heart disease and diabetes mellitus (WHO, 2016). Fruits and their derived products are rich source of numerous naturally occurring compounds such as phenolic acids (hydroxybenzoic and hydroxycinnamic acid derivatives), flavonoids and anthocyanins (Robards et al., 1999;. Wang, 2003). The fruit wine is one of the products which is possible to make from various types of fruit. It is important to emphasize that during fruit processing active principles retain in the final product (Czyzowska and Pogorzelski, 2002). Berry fruit wines are

[1]University of Belgrade, Faculty of Agriculture, Nemanjina 6, Beograd-Zemun,Serbia (aleksandar.petrovic@agrif.bg.ac.rs)

[2]Institute of Nuclear Sciences Vinča, University of Belgrade, National Institute of the Republic of Serbia, Mike Petrovića Alasa 12-14, Belgrade, Serbia

[3]University of Belgrade, Faculty of Pharmacy, Vojvode Stepe 450, Beograd, Serbia (urosc@phamacy.bg.ac.rs)

especially known for their good antioxidant and radical scavenging properties (Johnson et al., 2011). It is also interesting to highlight the natural active principles from fruit, which display α-glucosidase inhibitory activity. The α-glucosidase represents enzyme, which is involved in the final step of the carbohydrate digestion. Natural active principles which have α-glucosidase inhibitory activity express much lower side effects then synthetic ones, and can be used in the control of postprandial hyperglycemia (Xiao and Hogger, 2015;McDougall et al., 2005).

The aim of this study was to show how microvinification procedure influences on the content of selected phenolic compounds and antioxidant, as well as on free radical scavenging properties of the wine. Also, α-glucosidase inhibitory activity of lyophilized fruit wine samples was evaluated.

Materials and methods

Blueberries (*Vaccinium myrtilus*) were obtained from the region of Rudnik mountain, Serbia. Microvinification procedure was conducted in two sets, and the first step in both sets was fruit disintegration. In order to inhibit growth of unwanted bacterial populations during fermentation 10 g of $K_2S_2O_5$ 100/kg was added in the fruit must. Total soluble solids (expressed in °Brix) were measured in the fruit must of the first set. Aiming to increase total soluble solids of must up to 20.5°Brix, sugar was added in the second set. Within the aforementioned sets of the experiment, two subsets were performed. While the first sub-set included addition of 2 g/100 kg of enzymatic preparation glycosidase (EPG) (Enartis, Italy), the second one omitted its use. Both sub-sets were inoculated with pure culture of the selected commercial wine yeast (ICV D254, Lallemand, Canada (L), and Lievito Secco, Enartis, Italy (E)) both yeasts *Saccharomyces cerevisiae* strain) in the amount of 20 g/100 kg, respectively. Microvinification was conducted in barrels with pigeage system while alcohol fermentation was conducted on 20°C over the next 7 to 10 days. After fermentation, blueberry wine was separated from pomace by sedimentation, and kept at 12°C for the next six months, until further studies.

Total soluble solids (TSS, expressed in °Brix)and alcohol concentrationexpressed as volume (vol. %) were evaluated.

Solid-phase extraction (SPE) on Oasis HLB 6CC 200 mg cartridges (Waters, Milford, MA, USA) was applied to decrease the influence of the matrix during phenolics identification. UPLC/MS-MS analysis was performed using a Waters Acquity Ultra Performance H-Class System (Waters, Milford, MA, USA). Redox potential of the fruit wine samples was determined using the FRAP test (Benzie and Strain, 1996).The obtained results were expressed in mmol/L Fe^{2+}. Anti-DPPH radical activity of the fruit wine samples was evaluated as previously

described (Blois, 1958). The obtained results were expressed as a reciprocal value I (%) multiplied by 100. Total phenolic content (TPC) of the fruit wine samples was estimated by the Folin–Ciocalteu method using gallic acid as a standard (Woraratphoka et al., 2007). The results were expressed in mg/L of gallic acid equivalents (mg GAE/L).

Lyophilized wine samples were tested for α-glucosidase inhibitory activity using modified method (McCue et al., 2005). Acarbose was used as a positive control. The results were expressed as IC_{50} (µg/mL). The p value lower than 0.05, was considered as a significant.

Results and discussion

Physicochemical parameterTSS content can predict alcohol content in wine (Table 1).

After UPLC/MS-MS analysis of blueberry wine control samples were compared with those ones made with sugar and/or EPG (Table 2). Actually, the sugar content significantly affected the content of selected phenolic compounds ($p<0.05$). Higher sugar content before fermentation leads to more abundant alcohol content in the final product which improve the extraction of phenolic compounds ($p<0.05$).EPG liberates phenolics from the glycoside form. It ispossible to highlight that microvinification procedure significantly affects on the content of phenolic compounds. The highest content was found in the blueberry wine samples prepared with sugar and EPG. Furthermore, the samples prepared with sugar/without EPG were richer in phenolics than those without sugar and with or without EPG. Samples prepared without sugar/with EPG contained more phenolic compounds than Control. Different yeasts used in microvinification did not show any significance in content of phenolic compounds ($p>0.05$). The major phenolic acid of blueberry wines was chlorogenic acid (Table 2.). Hydroxycinnamic acid derivatives such as caffeic and *p*-coumaric acids were also identified which was according to literature data (Häkkinen et al., 1999; Zadernowski et al., 2005). Gallic acid was also detected in significant amounts.

Protocatechuic acid was the most abundant hydroxybenzoic acid derivative, as previously reported (Zadernowski et al., 2005). Additionally, Häkkinen et al. (1999) confirmed the presence of *p*-hydroxybenzoic acid in blueberries. Hydroxycinnamic were more abundant than hydroxybenzoic acid derivatives, as described before (Zadernowski et al., 2005). Epicatechin and catechin were also identified, as expected (Liwei et al., 2003). Blueberry wines were enriched with epicatechin (65.84µg mL-1) ($p < 0.05$). Such a finding is well supported by a Dutch study (Arts et al., 2000).

Table 1. TSS content of must and alcohol content in ine

Type of wine	Total soluble solids of must (°Brix)	Alcohol content in wine (Vol. %)
Control E.	14.67	7.97
+sugar− enzyme E.	18.48	9.81
−sugar+ enzyme E.	14.71	8.12
+sugar+ enzyme E.	18.75	10.51
Control L.	14.57	8.27
+sugar− enzyme L.	19.11	11.00
−sugar+ enzyme L.	14.77	8.45
+sugar+ enzyme L.	19.27	11.25

Control -sugar-EPG

Different experimental sets in microvinification showed the same trend for the antioxidants content assayed by TPC, FRAP and DPPH (Table 3.) just like UPLC/MS-MS analysis ($p<0.05$). The highest content of antioxidants was detected in the sample with sugar and EPG, while the lowest was in those without sugar and without EPG. Different yeasts used in microvinification did not show any significance for this three parameters ($p>0.05$). The FRAP and DPPH depended on the cumulative (synergistic) effect of various compounds present in fruit wines.

Table 2. The content of phenolic compounds in blueberry wine (μg/ml)

Type of wine	1	2	3	4	5	6	7	8
Control E.	725.15	92.47	1.71	54.12	75.23	2.27	32.18	42.51
+sugar - enzyme E.	775.57	120.51	3.41	65.43	114.72	4.82	42.83	60.45
−sugar +enzyme E.	738.23	97.81	2.31	52.31	79.51	3.88	37.81	41.27
+sugar +enzyme E.	797.47	127.87	4.27	72.71	118.89	7.81	47.17	63.42
Control L.	727.15	90.52	1.85	55.71	77.12	2.37	30.45	41.23
+sugar −enzyme L.	777.57	122.31	3.17	67.57	115.84	4.62	43.77	61.57
−sugar +enzyme L.	736.43	96.43	2.47	55.43	81.51	4.17	35.76	44.52
+sugar +enzyme L.	801.23	125.91	4.35	71.48	120.45	8.21	48.64	65.17

1-Chlorogenic acid; 2-Caffeic acid; 3-*p*-Coumaric acid; 4-Gallic acid; 5-Protocatehuic acid; 6-*p*-Hydroxybenzoic; 7-Catechin; 8- Epicatechin

The results for TPC, FRAP and DPPH depended from alcohol content which improved the extraction of phenolic compounds leading to the enhanced TPC, antioxidant and antiradical potential of the final product. EPG also contributed to more profound antioxidant and antiradical potential. Another study by Vasantha Rupasinghe and Clegg (2007) showed significantly profound redox potentials for blueberry which is in line with our findings.

Table 3. FRAP, TPC, DPPH and α-glucosidase inhibition values for blueberry wine samples

Type of wine	FRAP (mmol L^{-1} Fe^{2+})	TPC (mg GAE L^{-1})	DPPH (IC_{50})	α-glucosidase inhibition (µg/ml)
Control E.	68.15	2234.45	1.57	45.26
+sugar −enzyme E.	77.18	2473.57	1.35	41.52
−sugar +enzyme E.	72.65	2317.21	1.53	34.18
+sugar +enzyme E.	83.21	2581.37	1.27	31.27
Control L.	69.23	2245.51	1.55	43.81
+sugar −enzyme L.	76.81	2489.83	1.39	32.25
−sugar +enzyme L.	71.43	2375.53	1.52	40.45
+sugar +enzyme L.	82.65	2595.17	1.31	30.15

The α-glucosidase inhibitory activity showed the same trend as UPLC/MS-MS, TPC, antioxidant and antiradical analysis ($p<0.05$) (Table 3). Values for IC_{50} of fruit wines were obtained from sigmoidal-shaped inhibition curves. The results indicated that our samples possess good α-glucosidase inhibitory activity (Table 3). Such findings are supported by the study of blueberry extracts which displayed a significant α-glucosidase inhibitory activity (McDougall et al., 2005).

Conclusion

In summary, it is possible to highlight blueberry wine as an important source of naturally occurring antioxidants, which exhibit health-promoting properties. The findings also indicated that blueberry wine inhibit α-glucosidase, and may be potentially used as a functional (medicinal) food in the

prevention of diabetes mellitus and other chronic diseases. Hydroxybenzoic and hydroxycinnamic acid derivatives jointly with other active principles – both phenolics and non-phenolics are responsible for beneficial health effects of fruit wine. Moderate consumption of blueberry wine may be recommended as a part of healthy (well-balanced) diet.

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PLUM AS A RAW MATERIAL AND ITS INFLUENCE ON THE QUALITY OF BRANDY

Dragana Stanisavljević [1], Jovana Mihajlović[1], Ivan Nešović[1], Milica Stojanović[1], Dušica Ćirković[1], Violeta Mickovski Stefanović[2], Predrag Ilić[3], Dobrila Randjelović[1], Dragan Veličković[1], Zvonko Zlatanović[1]

Abstract: Proper selection and knowledge of varieties, processing conditions, and the use of selective yeast cultures during alcoholic fermentation have an important influence on the chemical composition of the distillate. In order to examine the influence of the plum variety on the quality of plum brandy, chemical analysis and sensory evaluation of brandies obtained from different varieties were performed. It was established that the characteristics of plum brandy depend primarily on the variety of plums. The best results were shown by brandies from the Moravka and Čačanska lepotica varieties, while the brandy sample from the Stanley variety was somewhat weaker.

Keywords: brandy, plum varieties, chemical composition, sensory characteristics

Introduction

We can safely say that šljivovica is a Serbian brand. In recent years, it has been an increasingly common practice to systematically create plum tree farms exclusively for the production of plum brandy (Popović et al., 2016). For the production of brandy from fruit in Serbia, brandy varieties of plum as well as varieties of plum with combined properties are most often used (Urošević, 2015). Požegača, Crvena ranka, Valjevka, Čačanska lepotica, produce top-quality brandy, as well as high-quality brandy (Nikićević, 2008). Some producers, due to steady fertility, decide to a certain extent when choosing a variety for the production of brandy to opt for the Stanley variety, although it produces brandy of mediocre quality (Popović et al., 2012). Research by many authors indicates the chemical and sensory properties of plum brandy depend

[1] Toplica Academy of Vocational Studies – Department of Agricultural and Food Studies, Ćirilo i Metodije 1, Prokuplje, Serbia
(draganastanisavljevic72@gmail.com);
[2] Tamiš Research and Development Institute, Novoseljanski put 33, Pančevo, Serbia;
[3] Faculty of Philosophy, Ćirila i Metodija 2, Niš, Serbia.

on the quality of the raw materials. The origin of the raw materials used for the production of fruit brandies has a great influence on the chemical content of the distillate (Madrera and Mangas, 2005). Popović et al. (2006) indicate that the quality of plum brandy, its chemical composition, and sensory characteristics are influenced by the plum variety and the technological process of production. An assessment of the influence of many factors on the quality of plums, such as climatic characteristics, soil characteristics, characteristics of the plum variety, and characteristics of the applied technological production process was also carried out (Miličević et al. 2012). When it comes to the production of top-quality fruit brandies, the fruit that represents the basic raw material in the production of such quality brandies must be technologically mature, undamaged, and well-preserved (Puškaš, 2011).

All volatile ingredients are not equally valuable for brandy quality, so the presence of some ingredients in brandy is desirable, while for others not as much. Klee (2010) states that volatile aromatic components important for the aroma and flavor of fruit are synthesized from different precursors (amino acids, membrane lipids, and carbohydrates). That is why it takes skill to conduct the distillation correctly and get a brandy with a pure smell of the fruit it comes from, a harmonious taste, and a fully clear liquid (Jović, 2006). Higher alcohols in distillates are usually found in quantities of 1.000 – 6.000 mg/L a.a. (Nikićević and Tešević, 2009). The potential amount of methanol in beverages depends partly on the content of pectin in the basic raw material and on the degree of its esterification. The potential amount of methanol in drinks also depends on the content of sugar in the basic raw material, that is, on its ratio to pectin (Nikićević and Tešević, 2005). Volatile aromatic esters form one of the larger groups of aromatic components in strong alcoholic beverages and they are responsible for the fruity note of the aromatic complexes (Urošević, 2015). Hydrocyanic acid and benzaldehyde are produced by the hydrolysis of amygdalin, which is found in the shell core and the seeds as well as by the hydrolysis of prunasin from the skin and flesh of stone fruits (Rajković and Perić, 2005).

The greatest importance, in addition to the chemical composition, of strong alcoholic beverages is attached to their sensory characteristics, as one of the most important quality factors. When descriptively evaluating the quality of strong alcoholic beverages, it is necessary to express sensory impressions as objectively as possible, whereby the training and practice of the evaluator greatly affect the degree of objectivity of the evaluation (Urošević, 2015). The research also has a practical significance for the production of plum brandy, so

it represents support for producers in the selection of raw materials, intending to obtain the highest quality product. The sample was also organoleptically tested and on that basis, its ultimate quality was determined. The goal of the research is to determine the differences in the quality of plum brandy, produced from different raw materials from the area of the municipality of Blace.

Materials and methods

Plum fruits grown in the territory of the municipality of Blace (distillery "Ivan Nešović PR Nešović produkt"), harvested in 2020, were used for the purposes of the test. The raw material used was at the stage of full technological maturity, which is optimal for the processing and production of plum brandy. Alcoholic fermentation was carried out in plastic vessels with raised pomace at a temperature of 20-25 °C. At the end of the fermentation, the distillation of the boiled mixture was started immediately, using a 400 L brick copper cauldron. The redistillation was carried out on the same device with the extraction of the first stream in the amount of 1%, the medium fraction up to an average strength of 60-65 vol%, and the stream fraction. The distillate was aged in new oak barrels with a volume of 200 L, after which the ethanol content in the samples was reduced to the desired amount with distilled water, which was taken for analysis.

The physical and chemical analysis of brandy was performed in the laboratory of the Toplica Academy of Vocational Studies (Department for Agricultural and Food Studies in Prokuplje). The following indicators were determined: ethanol concentration, extract content, total acid content, ester content, methanol content, higher alcohol content, total aldehyde content, furfural content, and benzaldehyde content, as well as the content of volatile ingredients, all according to the Rulebook on sampling methods and performing chemical and physical analyzes of alcoholic beverages ("Sl.list SFRJ" number 70/87).

The evaluation was done according to the method of 20 positive points and the evaluators expressed their observations about the sensory properties on the evaluation sheet by rounding the number for each sensory property in the column below each of the criteria. Sensory tests, scoring of characteristics such as color, clarity, typicality, smell, and taste, and then their sensory description, was done to obtain a complete picture of the quality of brandy. Scoring was done according to the following principle: color 0-1 point, clarity 0-1 point, typicality 0-2 points, smell 0-6 points and taste 0-10 points (Nikićević and Paunović, 2013).

Results and discussion

The studies included physicochemical and sensory analysis of brandy made from different varieties of plum. This paper presents analyzes performed on brandies produced from frequently grown plum varieties in the Republic of Serbia: Moravka, Čačanska lepotica, and Stanley. Table 1 presents the chemical parameters of plum brandy samples.

Table 1. Chemical composition of plum brandies

Chemical composition	Plum brandy (Moravka)	Plum brandy (Stanley)	Plum brandy (Čačanska lepotica)	Prescribed values
Ethanol % v/v	45.76	42.74	48.08	min. 37.5
Total extract g L^{-1}	0.36	0.42	0.25	
Total acids mg L^{-1} a.a	948	1124	790	
Esters mg L^{-1} a.a	1773	1617	1480	
Methanol mg L^{-1} a.a	3006	3560	2452	max. 12000
Higher alcohols mg L^{-1} a.a	1160	993	1618	
Furfural mg L^{-1} a.a.	9	13	5	
Aldehydes mg L^{-1} a.a.	92	99	88	
Benzaldehyde mg L^{-1} a.a	79	51	38	
Volatile ingredients mg L^{-1} a.a	3034	2722	3191	min. 2000

Brandies and other alcoholic beverages, before regulation and placement on the market, are subject to mandatory quality testing, under the Law on Strong Alcoholic Beverages. Brandies obtained from different varieties of plum show different results. The concentration of ethanol ranged from 42.74 to 48.08 vol%, which represents mostly the strength for this type of strong alcoholic drink, with a note that considering the abundance of plum brandy, an ethanol concentration of up to 45 vol% is sensory most suitable for this type of brandy. The content of total acids ranged from 790 to 1124 mg L^{-1} a.a.. The content of esters speaks of the well-defined aroma of the tested brandies and ranged from 1480 to 1773 mg L^{-1} a.a. and it was with the highest value in brandy from the

Moravka variety. The methanol content as a quality parameter was within the permitted limits. The highest methanol content was found in Stanley plum brandy (3560 mg L^{-1} a.a.), while the least amount of methanol was found in Čačanska lepotica plum brandy (2452 mg L^{-1} a.a.). Higher alcohols in the examined samples are within the limits of 993 mg L^{-1} a.a. up to 1618 mg L^{-1} a.a. The content of furfural in the examined samples was within the limits of 5 mg L^{-1} a.a. up to 13 mg L^{-1} a.a. Total aldehydes, depending on the variety, range from 88 to 99 mg L^{-1} a.a.. The highest values were recorded in the samples of brandy obtained from the Stanley plum variety. The benzaldehyde content ranged from 38 mg L^{-1} a.a. in the sample of brandy from the Čačanska lepotica variety, up to 79 mg L^{-1} a.a. with a sample of brandy from the Moravka variety.

The examined samples had a suitable amount of volatile components, i.e. the content of volatile ingredients was above the minimum value defined by the Law. As for the volatile ingredients, the difference in their quantity in these three brandies is negligible, especially between Moravka and Čačanska lepotica brandy, while Stanley plum brandy recorded a lower result of 2722 mg L^{-1} a.a.

The subject sample brandy from the Čačanska lepotica variety won 18.10 points, which is 90.50% of the maximum possible quality. The subject sample of Moravka brandy won 17.90 points, which is 89.50% of the maximum possible quality. The subject sample of Stanley brandy won 17.50 points, which is 87.50% of the maximum possible quality.

Table 2. Sensory evaluation of the plum brandies

	(Moravka)	(Stanley)	(Čačanska lepotica)
Average	17.90	17.50	18.10
Description	Brandy's color is yellow and clear. The smell is characteristic of the variety, intense, clean, nice, and pleasant. The taste is sweet, fruity, and pleasant.	Brandy's color is yellow and clear. The smell is characteristic of the variety, clean, medium-intense, and pleasant. The taste is light, fruity, and drinkable.	Brandy's color is yellow and clear. The smell is characteristic of the variety, clean, intense, and pleasant, a little sharp due to the alcohol content. The taste is full, rounded, and perfumey.

Conclusion

According to their chemical characteristics, the tested brandies fit within the framework of the current Law on Strong Alcoholic Beverages. The quality of plum brandy, its chemical composition, and its sensory characteristics are influenced by the type of plum and the technological process of production. It was established that the characteristics of plum brandy depend primarily on the plum variety. The content of ethanol and other parameters was within the limits of what was expected for the examined brandies and in accordance with the current law. There are certain differences depending on the use of appropriate varieties of plum, based on which we can more clearly see their influence on achieving the best possible quality. The samples of Čačanska lepotica and Moravka brandy were rated with high ratings of sensory characteristics, while the brandy of the Stanley variety was rated somewhat lower. These results unequivocally indicate the advantage of domestic plum varieties in the production of high-quality brandy.

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WATER COMPOSITION AND THEIR INFLUENCE ON THE WORK OF MALT AMYLOLYTIC ENZYMES

Dragana Stanisavljević [1], Aleksa Crkvenjakov[1], Jelica Lazić Saković[1], Nebojša Milosavljević[1], Svetlana Bogdanović[1], Violeta Mickovski Stefanović[2], Predrag Ilić[3], Jovan Ćirić[1], Dejan Davidović[1], Aleksandar Veličković[1]

Abstract: Water is an indispensable part of beer and brewing practice, and therefore it is not even possible to imagine a process in which water quality is neglected. For the purposes of this experiment, several different watersamples were collected from available springs. The basis of the experiment was the colored reaction of iodine on starch, which would prove the hydrolysis of starch granules from grains. This leads to the conclusion that the mineral composition, which affects the hardness of the water, does not have a decisive influence on the work of amylolytic enzymes, at least not to the extent that would significantly affect the production process.

Keywords: water, beer, amylolytic enzymes, pH value

Introduction

As one of the most widespread and consumed beverages in the world, beer occupies a special place in human society. A small percentage of alcohol, a pleasantly sharp and bitter taste, as well as attractive packaging, made it extremely popular all over the world and in all eras, so much so that this drink has become an indispensable part of many cultures and economies around the world. The nutritional importance of beer is great. It is rich in nutrients, carbohydrates, amino acids, minerals, vitamins, and phenolic compounds (Gerhäuser, 2005). Beer also contains 5-10 mg/l of soluble crude fibers, which come from malt (Leskošek-Čukalović, 2002). The medicinal properties of beer have been confirmed in many works (Leskošek-Čukalović, 2016). Beer has a beneficial effect on the psyche, thus promoting a good mood, relaxing, and reducing stress (The Brewers of Europe, 2008).

[1]Toplica Academy of Vocational Studies – Department of Agricultural and Food Studies, Ćirilo i Metodije 1, Prokuplje, Serbia
(draganastanisavljevic72@gmail.com)
[2] Tamiš Research and Development Institute, Novoseljanski put 33, Pančevo, Serbia
[3]. Faculty of Philosophy, Ćirila i Metodija 2, Niš, Serbia.

Modern brewing is characterized by the use of four raw materials: *water, malt, hops, and yeast* (Leskošek-Čukalović, 2004; Pejin, 2019). The quality of all raw materials in the production of beer has a decisive influence, even often decisive, on the quality of the finished product - beer (Grujić et al., 2000). The quality, that is, the composition of water, affects the production of beer in many ways, and it is most noticeable during the extraction of soluble substances from grains, and the enzymatic reactions that occur on that occasion. Malt has a decisive influence on the quality and taste of beer, as well as on the price of the final product. Hops (lat. *humulus lupulus*) is a perennial creeping plant whose female cones are used in brewing. The lupulin particles located under the leaves of the cones contain substances that are isomerized during the cooking of the wort and become bitter and more soluble, which contributes to the characteristic taste of beer. The process of isomerization produces isohumulone, which is soluble in wort and to the greatest extent causes the bitterness of beer (Kunze, 2010). The optimum temperature for most top-fermenting yeasts ranges from 15 to 20°C, while for bottom-fermenting yeasts it is several degrees lower (Boulton and Quain, 2001).

Materials and methods

For the purposes of this experiment, several different water samples were collected from available springs. Care was taken to ensure that the collected samples were appealing either as sources of existing breweries (Drava, Nišava) or that the sources were characteristic on other grounds (lake or well water). The necessary analyzes were performed in the laboratory of the Toplica Academy of Vocational Studies (Department for Agricultural and Food Studies in Prokuplje).

The pH value was measured using an immersion electric pH meter at a temperature of 22°C. Conductivity was determined using an immersion conductometer. Total and carbonate hardness were measured using volumetric methods, while non-carbonate hardness was determined computationally from their difference.

The malt used in the experiment is light pilsner-type malt, obtained from double-row barley of the Nectaria variety from the "Maltineks" malthouse near Bačka Palanka. The basis of the experiment was the colored reaction of iodine on starch, which would prove the hydrolysis of starch granules from grains. Amylose in starch causes the appearance of a dark blue color in contact with iodine. The preparation of the experiment entailed crushing the malt in order to expose the starch granules to water. Since the filterability of the pomace was not important, attention was not paid to preserving the membrane grain, as in commercial production, but using a mixer with blades, at 1000 revolutions per minute for 20

seconds, the malt was crushed to a fine granulation. 40 grams of crushed malt was poured into 6 laboratory beakers of 250 milliliters each, and 120 milliliters of sampled water preheated to 80°C was added to each. After homogenization of the mixture, the glasses with the samples were placed in a water bath at a temperature of 72°C. Every 5 minutes, the samples were mixed while checking the temperature and reaction to iodine. To check the iodine reaction, a few drops of pomace were poured onto a white porcelain surface, to which 2 to 3 drops of iodine solution would then be added. The appearance of a dark color would indicate the presence of iodine. After the last occurrence of negative iodine reactions, the pH values of the samples were measured using an electric pH meter.

Results and discussion

Malt analysis shows the following quality:

Table 1. – Malt specification (obtained from the malthouse)

Extract, dry matter [%]	> 81
Moisture [%]	< 5
Malt color [EBC]	3-4
Protein content, dry matter [%]	9.5 - 11.5
Kolbach index [%]	38 - 45
Viscosity [mPas]	5.8 - 6.0
β – glucans [mg/l]	< 250

The quality analysis of the samples can be seen in the following table 2:

Table 2. Water sample analysis

Water sample	pH	Conductivity [μS/m]	Total hardness [°N]	Carbonate hardness [°N]	Non-carbonate hardness [°N]
Danube (Kovilj)	7.47	428	14.3	12.4	1.9
Drava (Osijek)	7.19	736	18.8	27.1	-8.3
Danube (Borča)	6.53	389	12.5	10.8	1.7
Nišava (Niš)	6.99	343	16.6	14	2.6
Bresnica (Prokuplje)	6.59	130	3.8	3.3	0.5
Distilled	6.45	5.7	0	0	0

Carbonate hardness was established to be larger than total hardness in the sampled water from the Drava river so a negative non-carbonate hardness value was obtained. Such waters are said to have "negative" hardness. This phenomenon is common with waters that have more alkaline hydrogen carbonates, i.e. sodium and/or potassium hydrogen carbonate. In this case, during the titration of water with a strong mineral acid, all bicarbonate ions participate, and not only those that are in balance with the cations that make up the hardness (Ca and Mg) (Anonymous, 2016). Waters where the situation is opposite (mval/l) have no non-carbonate hardness (Gaćeša, 2000).

Table 3 shows the measured pH values of the pomace samples.

Table 3. Value of pomace samples – pH

Pomace sample	pH
Kovilj	5.21
Osijek	5.34
Borča	5.15
Niš	5.24
Prokuplje	5.33
Control	5.06

Most of the pomace samples had, from the aspect of amylolytic enzyme activity, a pH similar to the optimum in the range of 5.4 to 5.6, which can be attributed to the joint action of the present minerals and malt ingredients acting as buffers (Narziss, 1988). A notable exception is the control sample, i.e. the pomace made with distilled water, where the increased acidity can be attributed to the absence of base ions that would neutralize part of the acid ions originating from the malt.

Table 4. – Time of negative iodine reaction

Sample/ t[min]	5	10	15	20	25	30	35	40	45
Kovilj	-	-	-	-	-	-/+	+/-	+/-	+
Osijek	-	-	-	-	-	-/+	+		
Borča	-	-	-	-	-/+	+/-	+		
Niš	-	-	-	-	-	-	+/-	+	
Prokuplje	-	-	-	-	-/+	+/-	+		
Control	-	-	-	-	-	+/-	+/-	+	

The beginnings of the saccharification process in the attached Table 4 are marked with -/+, i.e. +/-, where a change in the color of the iodine reaction from dark to light could be observed, but it was not complete. The sign + indicates the moment when a negative reaction to starch definitely occurred.

As can be seen, most of the samples showed similar saccharification times, with a range of only 5 minutes between most samples. This leads to the conclusion that the mineral composition, which affects the hardness of the water, does not have a decisive influence on the work of amylolytic enzymes, at least not to the extent that would significantly affect the work of the production process. The only exception in this experiment is the water sample from Kovilj, which took the longest time to reach complete saccharification. This may be related to the fact it had the highest initial pH of all samples, although the origin of this elevated basicity remains unexplored for now.

Conclusion

The results of the experiment point to several conclusions. Although it is already known that the mineral composition of water has an influence on the work of amylolytic enzymes, it was not known whether these specific samples show greater mutual differences under the given conditions. Considering the geographical range of collected samples, only one showed a greater deviation in terms of composition (Bresnica), but the results of the experiment showed that it did not have a greater impact on the enzyme's work. Other minerals may have a greater influence on their work, but the question remains in what kind of waters they would be found in noticeable quantities. It is likely that the variation of other factors during fermentation, such as temperature, would have an individually greater influence on enzyme activity than the composition of the investigated waters. From the practical aspect of the application of this information in the plant, it can be concluded that it is not crucial to use complex systems for softening water, or other preparation, if it meets the condition of hygienic correctness. Also, the question of the strategic position for opening a brewery, in terms of the availability of water sources of a certain quality, is secondary if the condition of water quality that meets the condition of hygienic correctness is met. Of course, some other water supply factors may influence the issue of building a new brewery, but that is a topic for another paper. Ultimately, the water samples used in the experiment yielded pomace with a pH value lower than optimal, so it is possible to achieve faster saccharification times, and thus savings in the plant if the water was treated so that it had a higher initial pH value. Water is an indispensable part of beer and brewing practice, and therefore it is not even possible to imagine a process in which water quality is neglected. This experiment, although small in scale, clearly shows that there is a lot about water that needs to be mastered in order to be able to confidently approach beer production.

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NUTRITIONAL VALUE OF COLD-PRESSED SUNFLOWER OIL

Aleksandra Stojićević[1], Biljana Rabrenović[2], Mališa Antić[2]

Abstract: The aims of this study were to investigate nutritional value of cold-pressed sunflower oil produced in the Republic of Serbia. Composition of fatty acids was determinated and, based on individual fatty acid content, nutritional indices are calculated. Tocopherol composition and total content of carotenoids and chlorophylls were investigated as well. The results indicate that examined oil is rich in essential linoleic acid and with excellent values of nutritional indices. Unfavorable n-6/n-3 ratio is caused by low n-3 fatty acids content which is characteristic for sunflower oil. Total tocopherol content was unexpected low while content of pigments was characteristic for this type of oil.

Keywords: cold-pressed sunflower oil, nutritional quality indices, tocopherols, carotenoids, chlorophylls

Introduction

Cold-pressed oils take a special part of the human diet because they are a significant source of essential fatty acids and bioactive compounds. Cold pressing as a method of production of oil preserve valuable components, such as vitamins, provitamins, phytosterols and phospholipids, which are together with some fatty acids, key factors of nutritional value (Rabrenović et al., 2014).

In our country the most commonly consumed oil is sunflower oil. The standard type of sunflower oil is characterized by a high proportion of polyunsaturated fatty acids (PUFAs) of which linoleic ($C_{18:2}$, n-6) is predominant. The amount of this essential fatty acid in the total fatty acid composition ranges from 55 to 75% (Romanić, 2020). The presence of linoleic acid in this amount makes sunflower oil nutritionally valuable and suitable from the healthy nutrition point of view. Besides energy, as well as structural role of PUFAs, linoleic acid gives rise to arachidonic acid, which is the major precursor of bioactive compounds – eicosanoids, which regulate a large number of physiological processes (Sanders, 2016). The health benefit of linoleic acid manifests by decreasing the level of low-density lipoprotein (LDL) cholesterol,

[1] Academy of Applied Technical Studies Belgrade, College of Professional Technical Studies Požarevac, Nemanjina 2, 12000 Požarevac, Serbia (astojicevic@atssb.edu.rs)
[2]Faculty of Agriculture, Nemanjina 6, 11080, Belgrade, Serbia

so an optimal intake of linoleic acid reduces the possibility of developing cardiovascular diseases (Ulbricht and Southgate, 1991). However, large intake also decrease the values of high-density lipoprotein (HDL) cholesterol. Intake of linoleic acid less than 1% is considered as deficit, which rarely occurs and results in poor growth and development in infants, scaly dermatitis and impaired immune response and reproduction (Sánchez-Muniz et al., 2016; Sanders, 2016).

The main representative of monounsaturated fatty acids (MUFA) in standard type of sunflower oil is oleic fatty acid ($C_{18:1}$, n-9) which generally ranges from 14 to 40%. Saturated fatty acids (SFA) are present with 8-15% of which palmitic acid ($C_{16:0}$) and stearic acid ($C_{18:0}$) are the most represented. Linolenic acid ($C_{18:3}$), n-3 essential fatty acid, in sunflower oil is present in negligible amounts (Romanić, 2020).

The main determinant of the quality, nutritional value and oxidative stability of edible oils is composition of individual fatty acids. The quality of dietary fats depends on their level of SFA, MUFA and PUFA and especially on the ratio of n-6 and n-3 fatty acids (Ratusz et al., 2018; Ying et al., 2018). The PUFA/SFA ratio is often used as an indicator of the atherogenicity of a fat or oil. Lipid disorders are of fundamental importance for atherogenesis as well as the occurrence of ischemic heart disease and other cardio- and cerebrovascular diseases. Atherosclerosis is caused by changes in the wall of blood vessels, characterized by the deposition of lipids from plasma lipoproteins. Among the long-chain SFA, myristic acid is fatty acid with the most pronounced atherogenic effect. Stearic acid and short-chain SFA (with ten or below carbon atoms) do not affect blood cholesterol. On the other hand, MUFAs and PUFAs of the n-3 and n-6 series, show neither an atherogenic nor a thrombogenic effect. Therefore, Ulbricht and Southgate (1991) proposed to include in the PUFA/SFA ratio not only atherogenic SFA but also MUFAs. Thus, a more reliable nutritional indice is obtained - the atherogenic index (AI). Also, the authors suggested an inverse ratio (SFA/PUFA) so that the AI would be the highest for the most atherogenic dietary components. This nutritional indice is calculated according to formula 1:

$$AI=(C_{12:0} + 4\times C_{14:0} + C_{16:0}) / [\Sigma MUFA + \Sigma(n\text{-}6) + \Sigma(n\text{-}3)] \quad [1]$$

Another important pathological process that results in cardiovascular diseases and is linked to fatty acid intake disorder is thrombosis. Ulbricht and Southgate (1991) also proposed another nutritional indicator – the

thrombogenic index (TI) which as well represents a modified PUFA/SFA ratio (formula 2). All thrombogenic SFAs (myristic, palmitic and stearic acid), MUFAs and PUFAs that show an antithrombogenic effect as well as the ratio of PUFAs from the n-3 and n-6 series are included in the calculation.

$$TI=(C_{14:0}+C_{16:0}+C_{18:0}) / [0.5\times\Sigma MUFA+0.5\times\Sigma(n\text{-}6)+3\times\Sigma(n\text{-}3)+(n\text{-}3)/(n\text{-}6)] \quad [2]$$

Based on the ratio of fatty acids that have a hypocholesterolemic effect to those that exhibit a hypercholesterolemic effect, Santos-Silva et al. (2002) proposed third significant nutritional indice - hypocholesterol/hypercholesterol index (HH) which is calculated according to the formula 3:

$$HH=[C_{18:1(cis9)}+C_{18:2(n\text{-}6)}+C_{20:4(n\text{-}6)}+C_{18:3(n\text{-}3)}+C_{20:5(n\text{-}3)}+C_{22:5(n\text{-}3)}+C_{22:6(n\text{-}3)}] / (C_{14:0}+C_{16:0}) \quad [3]$$

Although the composition of fatty acids has the greatest effect on nutritional value, minor components also affect even if they are present in a small amounts. Vegetable oils obtained by the cold pressing method are characterized by a high level of naturally present non-glyceride components such as tocopherols, carotenoids and chlorophylls. Sunflower oil, especially cold-pressed, is one of the richest sources of tocopherols, which content varies depending on the growing condiditons of the plant from which the oil is extracted, as well as on the processing and storage conditions. With 95-98% of total tocopherol content, main isomer in sunflower oil is α-tocopherol. High concentration of this isomer in tocopherol composition, makes sunflower oil the richest source of vitamin E among all commercially available oils (Romanić, 2020). Further, a high amount of α-tocopherol can be used as an indicator of the purity of sunflower oil (Kostadinović Veličkovska et al., 2015). In the human body they act as strong antioxidants which prevent oxidative, proliferative, metabolic ad inflammatory damage at the cellular level and the subsequent development of various chronic diseases (Romanić, 2020).

Carotenoids and chlorophylls are the most abundant pigments in edible oils. Sunflower oil pigments mainly consist of two carotenoid groups – xanthophylls and dihydroxy carotenoids (principally lutein), which comprise 76-81% of the total carotenoids present in oil (Rade et al., 2004). Some of caroteoids, such as α-caroten, β-caroten i β-criptoxantin are precursors of vitamin A while others do not have provitamin activity. Also, some carotenoids act as an antioxidant. Low carotenoid intake believe to increase risk of cancer,

skin damage and cardiovascular diseases (Górnaś et al., 2016). There is also some evidence of a beneficial effect on cognitive function (Eggersdorfer and Wyss, 2018).

Chlorophylls are green pigments present in CPSO in very small amounts, while they are almost absent in refined oil. Chlorophylls and their degradation products act like strong prooxidants in the presence of light and atmospheric $^3O_2$ thus accelerate the oxidation of the oil. In some cases, they can act as antioxidants.

The aim of this study was to analyze some nutritional characteristics of CPSO produced in the Republic of Serbia. In that aspect, composition and content of fatty acids were investigated and, based on obtained results, nutritional quality indices (AI, TI and HH indexes) were calculated. The total content of tocopherols, carotenoids and chlorophylls were also analyzed.

Materials and methods

Fresh cold-pressed sunflower oil (CPSO) was obtained from Uvita D.O.O. company (Debeljača, Serbia) and stored in the dark in original packaging before analysis. The fatty acid composition was determined by gas chromatography – mass spectrometry (GC-MS) method according to standard procedures (SRPS EN ISO 12966-1:2015; SRPS EN ISO 12966-2:2017). The fatty acids content was calculated in mg g^{-1} of oil and expressed, in relative quantity, as a mass percentage of the total content of fatty acids. Nutritional quality indices (AI, TI and HH) were calculated on the basis of individual fatty acids content according to formulas 1, 2 and 3 given above in the text.

Analysis of tocopherols was performed using high-performance liquid chromatography (HPLC) as described in the literature (Rabrenović et al., 2014). The results of the tocopherol content were expressed in mg $100g^{-1}$ of oil, whereby β- and γ-tocopherol isomers were shown collectively.

The content of total carotenoids was determined by the spectrophotometric method according to Górnaś et al. (2016) and expressed in mg kg^{-1} of oil. The content of total chlorophylls was determined according to the method provided by Pokorný et al. (1995) and expressed in mg of pheophytin *a* kg^{-1} of oil.

Results and discussion

The composition and content of fatty acids in analyzed oil is shown in Table 1. Unsaturated fatty acids were predominant (95% of the total). PUFAs were the main group in fatty acid composition with 77.40%, while MUFAs were present with 17.60%. The amount of SFAs was 5,50%, mainly consisting of palmitic (3.0%) and stearic acid (1.9%). Linoleic acid was dominant with 75.8% followed by oleic acid (16.4%). Fatty acids from n-3 series were found in traces which gives unfavorable n-3/n-6 ratio. The optimal n-3/n-6 ratio is important to provide cardio-vascular health benefits (Konuskan et al., 2019).

Table 1 Fatty acid composition of analyzed CPSO

Fatty acid		(% w/w)
Palmitic	C16:0	3.0
Palmitoleic	C16:1	0.1
cis-10-heptadecenoic	C17:1	0.3
Stearic	C18:0	1.9
Oleic	C18:1	16.4
Elaidic	C18:1	0.8
Linoleic	C18:2	75.8
Linolelaidic	C18:2	0.8
γ-linolenic (GLA)	C18:3	0.1
α- linolenic (ALA)	C18:3	0.2
Dihomo-γ-linolenic (DGLA)	C20:3	0.4
Eicosapentaenoic (EPA)	C20:5	0.1
Tricosanoic	C23:0	0.5
Lignoceric	C24:0	0.1
SFA		5.50
MUFA		17.60
PUFA		77.40
AI		0.03
TI		0.10
HH		31.10

Table 1 also shown calculated nutritional quality indices - atherogenic (AI), thrombogenic (TI) and hypocholesterol/hypercholesterol indexes (HH). Lower AI and TI values and higher HH values indicate high nutritional quality of the oil. The AI value was 0.03, which indicates a high antiatherogenic effect of tested CPSO. Ulbricht and Southgate (1991) for sunflower oil reported a slightly

higher value - 0.07. Ying et al. (2018) obtained 0.05 for AI values for wild caraway oil and apricot kernel oil.

The TI value was 0.10. According to different authors, sunflower oil had TI values from 0.19 to 0.28 (Ulbricht and Southgate, 1991; Đurović et al., 2021). Comparable values of this nutritional indice have wild flax and apricot kernel oil (0.10 and 0.11, respectively) (Ulbricht and Southgate, 1991; Ying et al., 2018).

The value of the HH index of the tested oil was 31.10. Based on the literature survey, this value is significantly higher than the values of various type of oil. According to Đurović et al. (2021), Omegol (mixture of refined rapeseed oil, sunflower oil and corn germ oil) which is only commercially available oil on the domestic market with a favorable ratio n-3/n-6 ratio, had 23.26 for this nutritional indice.

The results of tocopherol, carotenoids and chlorophylls content of the analyzed oil are shown in Table 2. The total tocopherol content was 22.05 mg $100g^{-1}$ of the oil. The dominant isomer was α-tocopherol (21.47 mg $100g^{-1}$), which represents 97.40% of the total detected tocopherols. The content of β- and γ-tocopherol fraction in the analyzed oil was 0.57 mg $100g^{-1}$. Since the proportion of β-tocopherol in sunflower oil is generally insignificant, the main isomer in this fraction is γ-tocopherol. δ-tocopherol, which presence is not typical for the standard sunflower oil, was not detected in the analyzed oil. The amount of total content of tocopherols is significantly lower than can be found in the literature for CPSO. Kostadinović Veličkovska et al. (2015; 2018) published slightly higher values (28.5 and 30.3 mg $100g^{-1}$). For CPSOs of different type of sunflower seed hybrids, Romanić (2015) reported range from 47.2 mg $100g^{-1}$ to 64.5 mg $100g^{-1}$, for total tocopherol content.

Table 2 Tocopherol composition and total carotenoids and chlorophylls content

Tocopherol content (mg $100g^{-1}$)	α-tocopherol	β + γ-tocopherol	δ-tocopherol	Σ
	21.47	0.57	-	22.05
Carotenoids (mg kg^{-1})	3.51 ± 0.16			
Chlorophylls (pheophytin *a* kg^{-1})	0.55 ± 0.03			

The total content of carotenoids in the examined oil was 3.51 ± 0.16 mg kg^{-1}, which is comparable with other published data (Romanić, 2015; Konuskan et al., 2019). The literature survey showed significantly higher amounts which often exceed 11 mg kg^{-1} (Premović et al, 2010; Tauferova et al. (2021). Lower values were reported by Rade et al. (2004), Topkafa et al. (2013) and Nezirević-Nizić et al. (2019).

The chlorophylls content was 0.55 ± 0.03 mg kg^{-1} expressed as pheophytin *a*, which is in agreement with the literature data (Premović et al., 2010; Topkafa et al., 2013; Romanić, 2015). Significantly higher content of chlorophylls was reported by Nezirević-Nizić et al. (2019) and Tauferova et al. (2021) (3.24 mg kg^{-1} and 2,53 mg kg^{1}, respectively), while Rade et al. (2004) for industrial obtained CPSO published 0.36 mg kg^{-1} for the content of total chlorophylls.

Conclusion

The results of this research clearly show that several factors must be included for the overall characterization of the nutritional value of edible oil. Tested cold-pressed sunflower oil is rich in essential linoleic acid and an excellent value of nutritional indices and optimal content of carotenoids. Unfavorable n-6/n-3 ratio was caused by negligible amounts of n-3 essential fatty acid, which is characteristic of sunflower oil. Besides this fact, nutritional characteristic of tested oil was decreased with a very low portion of tocopherols. Improving the nutritional value of this oil, in aspect of increasing n-3 essential fatty acid content, can be performed by blending it with linolenic acid-rich oil such as camelina or flaxseed oil.

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SENSORY PROPERTIES OF HOMEMADE AND INDUSTRIAL MAYONNAISE

Jasmina Tahmaz[1], Amra Sejfić, Enver Karahmet[1], Sabina Operta[1], Senita Isaković[2]

Abstract: The aim of this study was to investigate sensory properties of industrial and homemade mayonnaise. Results showed that homamade mayonnaise contained higher level of oil, NaCl and egg yolk. Industrial mayonnaise had slightly higher scores for all sensory properties and overall acceptability. Mayonnaise samples have scores between 5.21 and 8.57 with no samples rated as unacceptable. Homemade mayonnaise vs. industrial samples had following average values: fat 71.08% vs. 67.20%, egg yolk 18.28 vs. 4.74% and NaCl 2.17% vs. 1.17%, overall acceptability scores 6.90 vs. 7.31. The best sensory properties have Thomy classic mayonnaise and homemade sample with 250 mLof oil, 2 egg yolks and lemon juice.

Keywords: Mayonnaise, low fat, sensory properties, egg yolk, consistency

Introduction

Mayonnaise is cold sauce wide used with different food (eg. cooked and potatos, sendwiches), produced as emulsion of oil, egg yolk and vinegar or lemon juice with addition of mustard and salt. According to Codex Alimentarius (1995) product declared as mayonnaise contains no less than 65% of oil, while egg yolk content is not precised. Previous Codex standard (1989) required ≥78.5% of fat and ≥6% of egg yolk. According to regional standards the lowest oil content is 70% in Bosnia and Herzegovina (Pravilnik, 2011) and 75% in Serbia (Pravilnik, 2013). The highest allowed level of oil in light mayonnaise is 45% according to Bosnian standard and 40% according to Serbian standard. The lowest level of egg yolk is not prescribed in Bosnian and Serbian standards.

Materials and methods

Research was performed on 10 industrial (IM1- IM-10) and 10 homemade (HM1-HM10) mayonnaise samples (Table 1 and 2). For homemade mayonnaise

[1]University of Sarajevo; Faculty of Agriculture and Food Sciences, Zmaja od Bosne 8, Sarajevo, Bosnia and Herzegovina (contact person e-maiL j.tahmaz@ppf.unsa.ba)
[2]Pharmacy&Bio d.o.o., Šefika Loje 22, Sarajevo, Bosnia and Herzegovina

the following ingredients were used: sunflower oil (Bimal Brčko, B&H), fresh hen eggs (Farm Ribarići Gornja Bioča, B&H), fine mustard (Polimark, Serbia), alcohol vinegar 9% (Kisko, Meteor Group Croatia), lemon juice squizeed from fresh lemons, table salt (Solana Tuzla, B&H), crystal sugar (Agragold, Brčko B&H) and white pepper.

Table 1 Industrial mayonnaise samples

Sample	Commercial name, and producer
IM-1	Mayonnaise classic Dijamant, Zrenjanin Serbia
IM-2	Zvijezda Delicatess Mayonnaise, Zagreb Croatia
IM-3	Thomy delicates, Nestle Adriatic Group, surčin Serbia
IM-4	Thomy classic, Nestle Adriatic, Surčin, Serbia
IM-5	Hellmanns Real Mayonnaise
IM-6	Mayonnaise Remia Nederlands
IM-7	Hellmanns delicatess Unileaver, Budapest, Hungary
IM-8	Mayo leggera Senna Senna, Austria
IM-9	Omegol mayonnaise Zvijezda Zagreb, Croatia
IM-10	Zvijezda light mayonnaise; Zagreb, Croatia

Table 2 Homemade mayonnaise samples

Sample	Ingredients - Recipe
HM-1	Oil 300 mL, 2 egg yolks, mustard 4,5 g, vinegar 4 g, salt 4 g, sugar 4 g, pepper 2 g
HM 2	Oil 250 mL, 2 egg yolks, mustard 4.5 g, lemon juice 6 g, salt 4 g, sugar 4 g, pepper 2g
HM-3	Oil 250 mL, 2 egg yolks, mustard 4.5 g, vinegar 4 g, salt 4 g, sugar 4 g, pepper 2 g
HM-4	Oil 200 mL, 2 egg yolks, mustard 4.5 g,vinegar 4 g, salt 4 g, sugar 4 g, pepper 2g
HM-5	Oil 200 mL, 2 egg yolks, mustard 4.5 g, lemon juice 6 g, salt 4 g, sugar 4 g, pepper 2g
HM-6	Oil 125 mL, 2 egg yolks, mustard 4.5 g, lemon juice 6 g, salt 4 g, sugar 4 g, pepper 2g
HM-7	Oil 125 mL, 2 egg yolks, mustard 4.5 g ,vinegar 4 g, salt 4 g, sugar 4 g, pepper 2 g
HM-8	Oil 100 mL, 2 egg yolks, mustard 4.5 g,vinegar 4 g, salt 4 g, sugar 4 g,pepper 2g
HM-9	Oil 100 mL, 2 egg yolks, mustard 4.5 g, lemon juice 6 g, salt 4 g, sugar 4 g, pepper 2g
HM10	Oil 50 mL, 2 egg yolk, mustard 4,5 g, lemon juice 6 g, salt 4 g, sugar 4 g, pepper 2 g

Homemade mayonnaise was prepared with highest speed of kitchen mixer (Daewoo) and according to recipes (Table 2). Egg yolks were mixed with mustard and 1/3 of vinegar/lemon juice. In the beginning, oil was added drop-by-drop with constant stirring. After obtained stable consitency, oil was added in thin stream. Remaining vinegar/lemion juice was gradualy added during mixing. Prepared samples had stable consistency without phase separation. pH value was determinated using pH-meter (Metler Toledo), fat by Soxhlet extraction and NaCl by Mohr titration. Egg yolk content in homemade mayonnaise was calculated from

total weight of all ingredients. Declared data for egg yolk were used for industrial samples. Sensory evaluation was done using of 9-point hedonic scale with 35 panelists. ANOVA and Tukey test ($p \leq 0.05$) were used for testing of significant differences between individual samples in each group. T-test ($p \leq 0.05$) was used for comparison of average differences between homemade and industrial samples.

Table 3 Average composition of mayonnaise samples (average±stdev)

Sample	Fat (%)	Egg yolk (%)	NaCl (%)	pH
IM-1	78.50±2-12a	6.50	0.75±0.04d	3.97±0.06c
IM-2	80.07±2.02a	6.60	1.53±0.13ab	3.75±0.03d
IM-3	79.90±3.39a	6.00	0.72±0.18d	4.07±0.01bc
IM-4	81.85±0.78a	5.00	1.35±0.09b	3.95±0.05c
IM-5	78.86±1.358a	7.90	1.30±0.20bc	3.98±0.02bc
IM-6	68.20±0.71b	6.00	0.83±0.07cd	4.10±0.05ab
IM-7	66.90±1.70b	4.30	0.61±0.04b	4.22±0.02a
IM-8	48.93±2.56cd	4.50	1.85±0.19a	3.98±0.01bc
IM-9	49.45±1.20c	0.00	1.29±0.10bc	3.97±0.00c
IM-10	40.53±1.83d	2.50	1.47±0.10ab	4.00±0.00bc
Average:	67.20±15.15n.s.	4.74±2.08*	1.17±0.41*	4.00±0.12*
HM-1	83.96±0.28a	10.50±0.18f	1.29±0,10e	4.46±0.01b
HM-2	80.78±0.58b	11.55±0.54f	1.41±0,01e	4.36±0.04b
HM-3	81.49±0.52b	11.78±0-30ef	1.41±0,01e	4.14±0.01c
HM-4	77.80±0.47c	14.43±0.44de	1.70±0,02d	4.48±0.04b
HM-5	77.48±0.69c	14.04±0.57d	1.68±0,01d	4.37±0.04b
HM-6	67.85±0.57d	20.01±0.72c	2.36±0,02c	4.71±0.01a
HM-7	68.36±0.43d	20.66±0.51bc	2.38±0,01c	4.81±0.01a
HM-8	64.00±0.47e	23.13±0.57b	2,79±0,02b	4.45±0.02b
HM-9	62.87±0.52e	23.13±0.63b	2.74±0,02b	4.39±0.01b
HM-10	45.89±0.83f	33,.61±1.23a	3.97±0,04a	3.91±0.06d
Average:	71.08±1.66n.s.	18.28±7.26*	2.17±0,84*	4.41±0.26*

Different small letters in columns represent significant ($p \leq 0.05$) differences between samples
Symbol * represents significant ($p \leq 0.05$) differences (t-test) obtained between average values of homemade and industrial samples

Results and discussion

Composition of homemade and industrial mayonnaise is given in Table 3. All samples contained required level of oil according to Codex Alimentarius

(1995) and national standards (Pravilnik, 2011; Pravilnik, 2013). Homemade mayonnaise contained higher amount of fat and egg yolk in comparison to industrial. pH value and NaCl also were slightly higher in homemade samples. Higher value of pH in homemede samples could be explained with higher amount of egg yolk (Satriawan et al. 2022; Rukke and Schuller). Egg yolks commonly have pH 6.37–7.00 (Ayola Mathew et al. 2016).

Table 4 Results of sensory evaluation of mayonnaise samples (average±stdev)*

Sample	Apperance	Consistency	Smell	Taste	Overall acceptability
IM-1	8,09±0.66ab	4,79±1.21e	6,74±0.17c	7,21±0.83b	6,38±0.91d
IM-2	6,47±0.65c	7,79±1.02ab	8,89±0.32a	6,80±0.93bc	7,53±0.88b
IM-3	7,56±1.01b	7,21±1.28bc	8,53±0.50a	8,26±0.44a	7,85±0.81b
IM-4	8,49±0.66a	8,51±0.70a	8,57±0.61a	8,40±0.74a	8,57±0.56a
IM-5	6,57±1.79c	7,06±1.21bc	7,20±1.43bc	6,94±1.98b	7,09±1.40cd
IM-6	6,47±0.85c	6,91±1.54cd	6,74±1.67c	6,15±0.65c	6,59±1.56cd
IM-7	6,80±0.76c	7,65±1.62bc	8,47±0.56a	8,27±0.56a	7,56±1.06b
IM-8	6,66±0.68c	6,32±1.02d	8,62±0.77a	8,21±0.93a	7,59±1.00b
IM-9	6,29±0.52c	7,15±0.73bc	7,62±0.78a	6,15±1.00c	6,71±1.13cd
IM-10	6,44±1.31c	7,79±0.68ab	8,50±0.70a	6,09±0.95c	7,21±1.16bc
Average	6,98±0.77	7,12±1.01	7,99±0.83*	7,25±0.97	7,31±0.66
HM-1	7,00±2.41ab	6,03±1.64bc	7,54±1.42a	8,34±1.06a	7,14±1.70a
HM-2	7,43±1.36ab	7,71±1.38a	7,51±1.50a	7,74±1.25ab	7,83±1.25a
HM-3	6,83±1.93ab	6,65±1.53abc	5,57±1.14d	5,31±1.62d	6,97±2.02a
HM-4	6,94±1.83ab	6,94±1.33ab	7,03±1.34ab	6,91±1.85bc	7,09±1.04a
HM-5	6,60±1.60b	6,66±1.59abc	6,40±1.54abcd	6,86±1.29bc	7,40±1.03a
HM-6	7,20±1.78ab	7,51±1.56a	7,23±1.85a	6,66±2.39bc	7,00±1.50a
HM-7	8,14±1.06a	5,63±2.50cd	5,80±1.55cd	7,57±1.12ab	7,23±1.63a
HM-8	7,43±0.92ab	6,97±1.01ab	6,83±1.10abc	7,23±1.09ab	7,29±1.02a
HM-9	5,46±1.56cd	5,57±1.29cd	5,97±1.62bcd	5,69±1.62cd	5,83±1.43b
HM-10	5,16±1.63d	4,81±1.94d	4,81±2.10d	5,38±2.17d	5,21±1.41b
Average	6,82±0.90	6,45±0.92	6,47±0.91*	6,77±1.03	6,90±0.78

Different small letters in columns represent significant ($p \leq 0.05$) differences between samples
Symbol * represents significant ($p \leq 0.05$) differences (t-test) obtained between average values of homemade and industrial samples

According to literature mayonnaise commonly contains 72.00-77.46% of fat, 0.6-02% of salt and 6-12% of egg yolk. In low fat mayonnaise oil content ranges 15-65% (Metri-Ojeda et al., 2022; Rukke & Schuler, 2019, Pazhvand &

Khavarpour 2019; Amin et al., 2014; Pradhananga & Adhikari 2015; El-Bostany et al. 2011; Mousakhani & Goli 2021). T-test ($p \leq 0.05$) showed that significant differences between homemade and industrial samples occurred in pH value, egg yolk and NaCl content (Table 3).

Homemade mayonnaise had slightly lower scores for all sensory properties (Table 4). Generaly all homememade samples had more intensive yellow color because of higher egg yolk amount. HM-1 sample with 300ml of oil had semisolid consistency. The main reported disadvantages of homemade samples were sour or oily taste, vinegar smell and thick consistency. The consistency was thicker in samples with higher oil amount. Samples prepared with lemon juice had slightly lower scores for taste. All samples were evaluated from 5.21 (neutral opinion) to 8.57 (very acceptable). The best sensory properties were noticed in HM2 (250 ml of oil and lemon juice) and IM-4 (Thomy classic). Industrial mayonnaises had lighter color, thinner consistency, lower sourness and mild taste. T-test ($p \leq 0.05$) showed that significant differences occured only in average scores for smell. Results of sensory evaluation were in agreement to literature (El-Bostany et al. 2011; Pradhananga and Adhikari 2015, Amin et al. 2015; Flamminni et al. 2020; Pazhvad & Khavarpour 2019; Karas et al. 2002) where was reported that experimental or low fat mayonnaises had lower scores.

Conclusion

Results showed pretty large differences in composition between homemade and industrial mayonnaise. Values of all ingredients were in the required limits. Homemade samples contained higher amount of fat, egg yolk and salt. Industrial samples were slightly better evaluated for all sensory properties. Generaly, samples with higher amount of fat, had better sensory evaluation. Samples were mostly rated from moderately to very acceptable. Altough professional equipement wasn't used in homemade preparation all samples had stable consistency without oil separation, which can be explained by high amount of egg yolk in recipes. Results suggest that it is possible to make stable and acceptable mayonnaise in home conditions. Sample prepared with 250 ml of oil and lemon juice could be recommended for mayonnaise preparation.

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THE ANTIOXIDANT POTENTIAL OF CONVECTIVE AND MICROWAVE-DRIED RASPBERRIES

Valerija Pantelić[1], Nemanja Miletić[1], Vesna Milovanović[1], Igor Đurović[1], Marko Petković[1]

Abstract: The present study aimed to evaluate the effect of convective and microwave drying on bioactive compounds in raspberries (*Rubus idaeus* L.) cv. Willamette and Tulameen. Fruits were dehydrated in the convective dryer at temperatures 50 °C and 70 °C and microwave oven at power levels (90, and 240 W). The highest percentage of anthocyanin (11.6 %) and polyphenols (25 %) was retention in microwave-dried Willamette at 90 W. The microwave-dried Willamette at 90 W also had the highest antioxidant potential of 1.53 ± 0.26 mmol TE/100g dry matter.

Keywords: convective drying, microwave drying, raspberry, antioxidant potential.

Introduction

Raspberries are one of the essential fruits in Serbian agriculture. According to the Statistical Office of the Republic of Serbia, in 2022, realized production of raspberries was 114987 t. In Serbia, raspberries are being produced on 19703 ha. The area of Western Serbia is the main production center with 87 % of the total raspberry production in Serbia. The most abundant raspberry cultivars in Serbia are Willamette and Meeker (Petrovic and Leposavic, 2011).

Red raspberries (*Rubus idaeus* L.) are soft, juicy fruits with a specific flavor and high nutritional values. The bioactive compounds and natural antioxidants in raspberries improve human health (Miletić et al., 2015). The raspberry is a rich source of phenolics, including anthocyanins and ellagitannins, and other bioactive. Anthocyanins are the major contributors to the red color pigment in berry fruits (Frías-Moreno et al., 2021). Apart from anthocyanins and ellagitannins, other phenolic compounds include hydroxycinnamic acids (caffeic, p-coumaric, and ferulic acids), flavonols, and condensed tannins (Lebedev et al., 2022). Polyphenols protect the body from oxidative stress

[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia (p.valeria.19@gmail.com)

considered to be the standard mechanism for the occurrence and progression of the most widespread chronic diseases, thereby contributing to the prevention of cardiovascular, cancer, and inflammatory diseases. The concentrations of the bioactive compounds in the fruits are affected by environmental conditions and the genetic characteristics of the varieties (Burton-Freeman et al., 2016).

Fresh raspberries are highly perishable fruits and are frequently dehydrated so that they are available to consumers throughout the year (Si et al., 2015). During traditional convective drying, heated air flows over the surface of the wet material, transferring heat to the wet material. During heating, moisture evaporates from the surface of the material, and at the same time, through the interior of the material, moisture moves towards the evaporation surface. A microwave oven uses electromagnetic waves called microwaves. In conventional drying, moisture is initially flashed off from the surface and the remaining water diffuses slowly to the surface. Whereas, in microwave drying, heat is generated directly in the interior of the material, creating a higher heat transfer and thus a much faster temperature rise than in conventional heating (Kalla and Devaraju, 2017).

The aim of this research was to investigate the influence of convective and microwave drying the antioxidative potential of red raspberry Willamette and Tulameen varieties. Evaluated quality parameters included changes in total phenolic content, anthocyanin content, and antioxidant activity in fresh and dried samples.

Materials and methods

Collecting samples – raspberry varieties Willamette and Tulameen were collected in Dragačevo area, on a family farm (village Gornji Dubac 43°39′54″N 20°21′56″E, altitude 850 m a.s.l.) few hours to each experimet. Raspberries were grown under organic agricultural practise. The fruits were visually selected according to size, color, maturity level, with no mechanical injuries.

Drying processes – raspberry varieties Willamette and Tulameen were subjected to convective (CD) and microwave drying (MW), to the constant mass. Convective drying of raspberries was carried out in a convective dryer (Gorenje Food Dehydrator FDK 500GCW), at an air temperature of 50 °C and 70 °C. Microwave drying was performed at the power levels of 90 W, and 240 W in the microwave oven (Tesla Microwave oven MW2390MB).

Dry matter content (DM), solid soluble content (SSC), mineral matter content (ash, MMC), and pH value were analyzed by the AOAC method, respectively (1995, 1990a, 1990b).

Determination of Total Anthocyanins (TA), Phenolics (TP), and Antioxidant activity (AA) – the monomeric anthocyanin content was determined by the pH differential method (Giusti and Wrolstad, 2001). Pigment content was calculated as milligrams of cyanidin-3-glucoside equivalents/100 g of fruit dried matter (mg cyn-3-glu 100 g^{-1} DM), using an extinction coefficient of 26.900 L cm^{-1} mol^{-1} and molecular weight of 449,2 g mol^{-1}. The total phenolics in ethanol extracts of fresh and dried raspberries were determined by Folin–Ciocalteu spectrophotometric method (Singleton et al., 1999). The results are expressed in milligrams of gallic acid equivalents (GAE) per 100 g of fruit-dried matter (mg GAE 100 g^{-1} DM). The antioxidant activity of the extracts against ABTS radical scavening was determined according to Re et al. (1999) and the results were expressed as Trolox equivalent antioxidant capacity (mmol TE 100 g^{-1} DM).

Results and discussion

The CD of Willamette at temperatures of 50 °C and 70 °C occurred at 2923 min and 1870 min, and Tulameen at 6010 min and 2094 min, respectively. CD at 70 °C the volume of the raspberries was preserved, while the long drying time at 50 °C caused the raspberry's shrinkage. MD drying the Willamette at microwave powers of 90 W, and 240 W occurred at 254 min, and 88 min, respectively. Drying of these varieties at 90 W and 240 W lasted 232 min and 99 min, respectively. MD led to greater changes in fruit volume than CD.

Values of DM in fresh Willamette and Tulamen were 13.18 ± 0.19 and 15.29 ± 0.98 %, SSC 9.4 ± 0.17 and 11.4 ± 0.17 %, MMC 0.41 ± 0.01 and 0.36 ± 0.01 %, and pH 2.91 ± 0.03 and 2.66 ± 0.03, respectively. The comparative analysis of DM and SSC in these cultivars showed higher values in Tulameen than in Willamette. Analysis of the pH value of fruits showed slight variation among cultivars. The results of chemical properties in this study agree with reports by Miletić's and Anjo's reports (Miletić et al., 2012; Anjo et al., 2020).

The antioxidative properties of fresh and dehydrated raspberries are presented in Table 1. The antioxidative potential varies in raspberry cultivars and depended on methods of drying. TA and TP in fresh fruits of Willamette were significantly higher than in Tulameen.

Table 1. Total anthocyanin and total phenolic contents and the antioxidant capacities (ABTS assays)

	TA (mg 100^{-1} g^{-1} DM)		TP (mg 100^{-1} g^{-1} DM)		AA (mmol TE 100^{-1} g^{-1} DM)	
	Willamette	Tulameen	Willamette	Tulameen	Willamette	Tulameen
fresh fruit	654.13	346.57	1686.56 ± 199.57	1038,00 ± 269.39	8.32 ± 1.30	5.41 ± 0.42
50 °C	21.76	4.23	251.65 ± 8.36	47.44 ± 3.28	1.16 ± 0.16	0.59 ± 0.11
70 °C	63.99	20.18	425.32 ± 6.30	344.75 ± 13.02	1.14 ± 0.02	0.95 ± 0.21
90 W	76.13	5.90	432.34 ± 4.49	131.21 ± 27.49	1.53 ± 0.26	0.57 ± 0.07
240 W	26.32	15.05	241.41 ± 25.73	319.06 ± 62.84	0.91 ± 0.11	0.85 ± 0.00

High temperature, long drying time, and power of the microwave oven caused a huge degradation of bioactive compounds in the samples. Increasing the dehydration temperature in CD, the antioxidative potential increased, and as well as increasing the microwave power in MD, the antioxidative potential decreased. The samples with CD of fresh raspberries at T = 70 °C and MD at 90 W had the lowest degradation of TA and TP in both varieties, with the highest AA (0.95 ± 0.21 mmol TE 100^{-1} g^{-1} DM for CD on 70 °C, and 0.85 ± 0.00 mmol TE 100^{-1} g^{-1} DM for MD on 90 W). Raspberry fruits dried at high microwave powers showed better preservation of bioactive materials, probably due to the shorter drying time (Si et al., 2015). Although the TA and TP in fresh raspberries were higher or similar than in other studies, during CD there was a significant decrease in the content (Miletić et al., 2012; Stamenković et al., 2019). The long drying time of the Tulameen variety at a temperature of 50 °C led to almost complete degradation of these bioactive components. It can be noticed that the Tulameen variety is more sensitive to the drying process than the Willamette regarding the loss of bioactive components.

AA of raspberries was significantly affected by drying methods. The initial AA of fresh raspberries of Willamette varieties was higher than that of Tulameen and these results are in correlation with reports by Rodriguez and Miletić (Miletić et al., 2015; Rodrigues et al., 2019). The CD method of Willamette did not significantly affect AA, unlike the MD; the highest percentage of antioxidant retention was 18.4 % for MD dehydrated raspberry on 90 W (1.53 ± 0.26 mmol TE 100^{-1} g^{-1} DM). For Tulameen, the highest retention of antioxidants was 17.6 % (CD at 70 °C). The results show a significantly lower percentage of retention of antioxidants (Rodriguez et al., 2019). Increasing the dehydration temperature in CD, the AA increased, and as well as increasing the microwave power in MD, the AA decreased. A more precise change in the

antioxidant capacity will be clearer when the influence of the drying method on the content of flavonoids and other bioactive substances is analyzed.

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Conclusion

Both applied methods of raspberry drying, traditional convective and microwave, reduced the content of bioactive compounds with antioxidant potential. The microwave drying significantly reduced the drying time compared to convective drying. The microwave-dried Willamette at 90 W had the highest antioxidant potential of 1.53 ± 0.26 mmol TE 100^{-1} g^{-1} dry matter and the highest percentage of anthocyanin (11.6 %) and polyphenols (25 %) retention. The Tulameen variety proved to be extremely sensitive to both dehydration methods.

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ENERGY USAGE AND RASPBERRY CONVECTIVE AND MICROWAVE DRYING PARAMETERS

Valerija Pantelić[1], Nemanja Miletić[1], Vesna Milovanović[1], Igor Đurović[1], Marko Petković[1], Alexander Lukyanov[2], Vladimir Filipović[3]

Abstract: The drying experiments of microwave (MD) and convective (CD) dehydration of raspberries show a shorter drying time by 86–96 % and lower energy usage resulting in energy saving of 81–89 % for MD. The average drying ratio of raspberries increases by 3.5–19 times with the application of MD. Newton's, Modified Henderson, and Pabis's, Logarithmic models were successfully used to describe the drying kinetics of raspberries. Willamette and Tulameen varieties, dehydrated on 240 W MD, showed the shortest dehydration time, the minimum energy usage, and the most efficient diffusion.

Keywords: drying, convection, microwave, raspberry, energy.

Introduction

Raspberries are considered to be a very perishable commodity because their moisture content is higher than 80%. Drying is a suitable modification of post-harvest management (Misha et al., 2022).

Microwave drying is a fourth-generation, drying technology. The key benefit of microwave drying, compared to convective drying, is its ability to preserve the quality and nutrients of the dried product. This is because the microwave energy heats the product from the inside out, leading to a more uniform drying process and reduced exposure to high temperatures that can cause oxidation and nutrient loss (Bórquez et al., 2010). Microwave energy can penetrate deep into the product and quickly remove moisture, leading to faster drying times and improved efficiency (Rodriguez et al., 2017).

The objective is to find the drying model that uses the lowest energy during raspberry drying and to determine the behavior and parameters of the model that can be used to optimize the drying process.

[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia (p.valeria.19@gmail.com)
[2]Don State Technical University, Ploshchad' Gagarina 1, Rostov-on-Don, Rostov Oblast, Russia
[3]University of Novi Sad, Faculty of Technology, Cara Lazara 1, Novi Sad, Serbia

Materials and methods

Raspberry varieties the Willamette and Tulameen were grown under organic agricultural conditions, on a family farm (village Gornji Dubac 43°39′54″N, 20°21′56″E). Before the drying process, the raspberries were cleaned and washed and visually selected according to maturity level, size, and color, without mechanical damage.

Convective drying (CD) of raspberries was carried out in a dehydrator (Gorenje FDK500GCW, 380 W, air velocity of 7.9 m s^{-1}) at temperatures of 50 °C, and 70 °C at atmospheric pressure, to the constant weight (Petković et al., 2020). The microwave oven (Tesla MW2390MB 1250 W) was used for microwave drying (MD) at 90 W, and 240 W. The uncrushed raspberries were dehydrated in a thin layer with a mass load of 1.33 kg m^{-2} (100 g per tray). The moisture ratio (MR) is determined according to Eq. 1:

$$MR = \frac{M_t - M_e}{M_o - M_e} \quad (1)$$

M_t, M_o, and M_e are the moisture content for drying time t, the initial moisture content, and the equilibrium moisture content, respectively. M_e is usually a deficient parameter. The drying ratio (DR) is the total mass loss of dehydrated materials ($M_{i-1} - M_i$) between two consecutive measurements ($t_{i-1} - t_i$) on a defined tray (Eq. 2):

$$DR = \frac{M_{i-1} - M_i}{t_{i-1} - t_i} \quad (2)$$

Based on the shape of the fruit (sphere), the effective moisture diffusivity D_{eff} can be calculated by Eq. 3 and Fick's second law of diffusion (Petković et al., 2021):

$$MR = \frac{6}{\pi^2} \times \sum_{i=1}^{\infty} \frac{1}{J_0^2} \times e^{\frac{J_0^2 \times D_{eff}}{4 \times r^2}} \quad (3)$$

D_{eff} is the effective moisture diffusivity (m^2 s^{-1}), t is time (s), J_0 is the roots of the Bessel function, and r is the radius of the sphere. If the D_{eff} was constant in a relatively long drying period, Eq. 3 could be derived in ln(MR) = ln(a) – k × t. The relationship between ln(MR) and t is linear, and the slope is equal to the drying constant (k, Eq. 4):

$$k = -\frac{\pi^2 \times D_{eff}}{4 \times r^2} \quad (4)$$

An Arrhenius equation, Eq. 5 for CD, and Eq. 6 for MD, describes this effect over the energy of activation (E_a; Filipović et al., 2022):

$$D_{eff} = D_0 \times e^{\frac{E_a}{R \times T}} \quad (5)$$

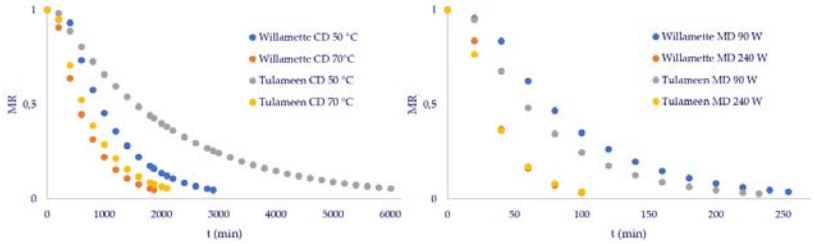
$$D_{eff} = D_0 \times e^{\frac{E_a \times m}{P}} \quad (6)$$

where E_a (kJ mol^{-1}) is the activation energy, R (8.3143 J $mol^{-1}K^{-1}$) is the universal gas constant, T (K) is the absolute air temperature, and D_0 (m^2 s^{-1}) is the pre-exponential factor of the Arrhenius equation. The Eq. 5 could be simplified in $\ln(D_{eff}) = \ln(D_0) - k \times (T + 273.15)^{-1}$. The relationship between $\ln(D_{eff})$ and T is linear, and the slope is equal to the drying constant $k = E_a \times R^{-1}$. The natural logarithm of D_{eff} versus mass load m (g) versus power P (W) was used to calculate the E_a (W g^{-1}) of MD, $\ln(D_{eff}) = \ln(D_0) - k \times m\ P^{-1}$. The plot is a straight line, and the slope is equal to the drying constant $k = E_a \times R^{-1}$ (Petković et al., 2022).

Energy usage (E) for CD and MD was measured by Prosto PM 001. The mathematical relationship between energy use and carbon dioxide emissions is 1 kWh = 0.998 kg CO_2.

Results and discussion

The initial dry matter content of the Willamette and the Tulameen raspberries was 13.18±0.19 % and 15.29±0.98 % (AOAC, 1995), respectively. The rate of diffusion through a material is proportional to the concentration gradient. The rate of diffusion is influenced by factors such as the size and shape/geometry of fruits, or the temperature or input power for drying (Bórquez et al., 2010). The fastest water removal occurred in the first (initial) stage of drying, regardless of CD or MD (Graph 1).



Graph 1. MR curves of CD and MD

With an increasing temperature of CD and the power of MR, the MR curves are steeper, so the drying process was reduced. At lower CD temperatures (50 °C) and lower MD power (90 W), the MR curves are steeper and the Willamette variety drying time was shorter. At higher temperatures (70 °C) and higher power (240 W), the drying time of the Tulameen was shorter. Due to a much-reduced drying rate in the second stage, the rate of water loss was slower (the MR curve was less steep). The shortest dehydration time had the Willamette on 240 W MW (88 minutes), and the longest dehydration time had the Tulameen at

50 °C CD (6010 minutes). With the increase in temperature and power, DR was grown, as well as the fact that it was greater at the Willamette variety. MD made the DR much higher, so the largest DR (1.933 g min^{-1}, obtained at 42nd minutes, Table 1) was noticed at the Willamette MD on 240 W, and the smallest DR (0.100 g min^{-1}, obtained at 173rd minutes) at the Tulameen at 50 °C CD. *Szadzińska* (2018) showed the drying ratio of CD (on 55 °C) was 0.04 g min^{-1} and 0.2 g min^{-1} (MD, 100 W). The MD assistance in the drying process could shorten drying time by 94 % (Mierzwa et al., 2019). With an increasing temperature of a CD and the power of MR, D_{eff} was increased. MD of the Tulameen on 240 W had the highest resistance to mass transfer (the maximum $D_{eff} = 1.17 \times 10^{-7}$ m^2 s^{-1}) and was about 25 times higher than the maximum D_{eff} of CD (4.43×10^{-9} m^2 s^{-1}, CD of the Tulameen on 70 °C; Table 1).

Table 1. Drying parameters and energy usage/CO_2 emission of CD and MD

	DR (g min^{-1})		D_{eff} (m^2 s^{-1})		E (kWh)		CO_2 (kg)	
	Willam.	Tulam.	Willam.	Tulam.	Willam.	Tulam.	Willam.	Tulam.
50 °C	0.100	0.067	3.75×10^{-10}	1.22×10^{-9}	6.215	6.533	6.203	6.520
70 °C	0.200	0.092	2.33×10^{-9}	4.43×10^{-9}	3.667	4.137	3.660	4.129
90 W	0.700	0.850	2.82×10^{-8}	4.40×10^{-8}	0.636	0.698	0.697	0.635
240 W	1.933	1.200	7.95×10^{-8}	1.17×10^{-7}	0.542	0.610	0.540	0.608

The fruit variety also affects the D_{eff} values and the drying parameters, as shown in our research. The D_{eff} ranged from 7.12×10^{-9} (CD, 65 °C) to 1.79×10^{-8} m^2 s^{-1} (MD, 450 W; Abbaspour-Gilandeh et al., 2020). The E_a shows the sensibility (the necessary energy required to begin the water diffusion) of the diffusivity against temperature and power range; the greater E_a means more sensibility of D_{eff} to temperature and power. Ea, for the CD, was calculated to be 69.38 kJ mol^{-1}, while for MW was 11.74 W g^{-1} (65.22 kJ mol^{-1}, the conversion factor between W g^{-1} and kJ mol^{-1} is: 1 W g^{-1} × M (1 g mol^{-1}) × (1 kJ (1000 J)$^{-1}$). Therefore, when CD and MD are compared, lower activation energy results in more effective moisture diffusivity (higher coefficient of mass transfer) and an increase in moisture diffusion with sphere diameter (the Tulameen's radius 25.0 mm, the Willamette's radius 21.6 mm), which results in a lower energy requirement. The E, as well as the emission of CO_2, was associated with the drying model and its kinetic parameters and variety, as well. The shortest drying time will have MD models with the highest power (240 W), as well as the highest D_{eff}, which was affected by the smallest E in the drying process 0.542–0.610

kWh (Table 1, the lowest emission of CO_2). The MD reduced energy consumption by 50 % (strawberry, Szadzińska et al., 2018).

CD and MD kinetic can be determined by fitting the MR values as a function of drying time to a mathematical model (Szadzińska et al., 2018). Several mathematical models could be used to describe the drying, including Newton's, Modified Henderson and Pabis's, Logarithmic model, etc. (Mierzwa et al., 2019; Table 2). All mathematical models for MR were found to be appropriate models for the thin-layer CD and MD of raspberries, according to the high values of the coefficient of determination (> 0.900). It could be noticed, regardless of the CD and MD, the increase in the coefficients in the exponent functions (k, g, h) with the increase in T and energy, i. e. the slope was shifted toward lower values of the moisture ratio and drying time. Other parameters (a, b, c) vary slightly within the limits. Also, under the same drying conditions, the coefficients of the mathematical models have lower values for the Tulameen compared to the Willamette variety.

Table 2. Values of the coefficients for mathematical models of CD and MD

Model	Newton $MR = e^{-kt}$	Modified Henderson and Pabis $MR = a\,e^{-kt} + b\,e^{-gt} + c\,e^{-ht}$						Logarithmic $MR = a\,e^{-kt} + c$		
	k	a	k	b	g	c	h	a	k	c
	Willamette									
50 °C	0.0007	0.3663	0.0007	0.3666	0.00076	0.3661	0.0007	2.0679	0.0003	1.02834
70 °C	0.0012	0.3586	0.0013	0.3589	0.00132	0.3582	0.0013	1.4163	0.0007	0.3966
90 W	0.0102	0.3809	0.0113	0.3703	0.01131	0.3703	0.0113	0.6791	0.0007	0.3209
240 W	0.0189	0.3986	0.0225	0.3986	0.02255	0.3986	0.0225	2.3650	0.0067	– 1.2462
	Tulameen									
50 °C	0.0006	0.3327	0.0004	0.3386	0.00043	0.3408	0.0004	0.6911	0,0002	0.3088
70 °C	0.0011	0.3548	0.0012	0.3553	0.0012	0.3549	0.0012	1.3075	0.0007	0.2833
90 W	0.0129	0.2231	0.0169	0.4727	0.0169	0.6335	0.0169	0.6525	0,0007	0.2709
240 W	0.0207	0.3539	0.0220	0.3541	0.0220	0.3527	0.0220	2.3487	0.0062	– 1.3357

Conclusion

The use of a microwave for drying raspberries is a more efficient method compared to conventional convective drying. The shorter drying time and lower energy usage in microwave drying result in significant energy savings. Furthermore, the results suggest that the microwave drying process leads to a higher drying ratio, meaning more water is removed from the raspberries during the drying process. The use of mathematical models such as Newton's, Modified

Henderson, and Pabis's Logarithmic models to describe the drying kinetics of raspberries shows that the drying process can be accurately predicted and modeled. The study also suggests that the Willamette and Tulameen varieties of raspberries showed the best results when dried using a 240 W microwave, in terms of shortest dehydration time, minimum energy usage, and most efficient diffusion.

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MOBILE DEVICE FOR CHEMICAL CLEANING OF HEAT EXCHANGERS

Tomislav Trišović [1], Branimir Grgur [2], Svetomir Milojević[3], Zaga Trišović [4]

Abstract: Heat exchangers are one of the most frequently used elements in the process industry. Water is usually used as a heating fluid and often is not chemically prepared, i.e. it has bicarbonate, calcium, and magnesium, which make the water temporarily hard and form incrustations on the primary side of the heat exchanger.

The paper shows a device for chemical cleaning of the heat exchanger without disassembling it and when the secondary side of the heat exchanger is actively working. Tests of the device in operation were carried out in the machine plant of the Tonanti Hotel in Vrnjačka Banja from 2021 to 2022, where it showed high reliability and complete independence in operation.

Keywords: heat exchangers, cleaning, descaling, cleaning of heat exchangers, heat pumps, heating and cooling.

Introduction

Cleaning the heat exchanger is often a big problem especially if the scale deposits are such that the specific heat flow through the exchanger is reduced and the pressure drop of the exchanger exceeds 0.5 bar. Then complete disassembly, mechanical and chemical cleaning of each part is necessary, which is an expensive and time-consuming process.

Another way is preventive cleaning of the exchanger after a certain period of operation, using acid-base means with equipment that is very expensive and fully automated. The liquid acid washing and disinfecting agent contains phosphoric and nitric acid, anti-corrosive additives, surfactants and sequestrants, and it is recommended to use it in combination with an alkaline

[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia (trisa@tmf.bg.ac.rs)

[2]University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, 11000 Belgrade, Serbia (BNGrgur@tmf.bg.ac.rs)

[3] University of Priština, Faculty of Technical Sciences, Knjaza Miloša 7, 38220 Kosovska Mitrovica, Serbia (svetomir.milojevic@pr.ac.rs)

[4] The Academy of Applied Technical Studies Belgrade, Katarine Ambrozić 3, 11000 Belgrade, Serbia (ztrisovic@atssb.edu.rs)

agent. The descaling agent is determined based on the chemical analysis of the water entering the heat exchanger. Based on the analysis, the composition of the acidic agent that will most successfully and quickly dissolve scale deposits is tested. When the type of acidic agent is determined, it is necessary to check the sealing of the vessel, ie. exchanger, by measuring the water pressure drop at the inlet and outlet of the heat exchanger. If the exchanger is passable, i.e. acidic medium can pass through the heat exchanger, the inhibited acid solution is dosed. During recirculation, the pH value of the solution is periodically measured. After cleaning, wastewater is neutralized.

Heat exchangers are often the heart of many processes in the food, pharmaceutical and other industries. Their regular maintenance and cleaning is of key importance for proper operation and a long working life.

Results and discussion

We designed and built a mobile device for chemical cleaning of heat exchangers with a simple construction and low cost, which can be used both in small plants and in larger facilities. The device has four components: an electromagnetic pump, a tank, a valve assembly with flexi pipes and a control cabinet. All elements are installed on a mobile platform that can be easily accessed by any exchanger that needs cleaning. Most components are made of plastic resistant to chemical agents. The device must not be in the vicinity of devices that spark or emit heat (grinders, welding devices, furnaces, etc.) during operation. The temperature range in the room where the device is located must be from 5 to 40 °C, air humidity from 20 to 80% and with no condensation. The electromagnetic pump serves to ensure the circulation of the mentioned acid with inhibitors from the tank through the heat exchanger and its return to the tank. Heat exchangers are usually connected via a bypass so that they can be easily disconnected from the main piping and serviced (Figure 1).

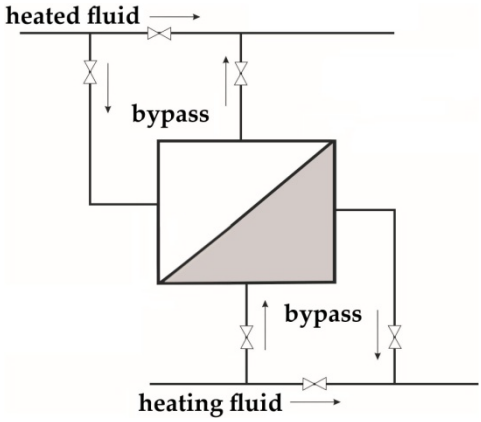

Figure 1. Block diagram of the connection of the heat exchanger

The heating fluid is of a higher temperature and it passes through the primary chamber of the heat exchanger, while the heated fluid is of a lower temperature and passes through the secondary part of the heat exchanger. When the primary or secondary bypass valves are closed, the heat exchanger can be serviced or dismantled. If thermal mineral water of high temperature is passed through the primary of the heat exchanger, it is possible to create scale deposits that reduce the efficiency of the heat exchanger or further scale deposition by completely closing the water passage on the primary side of the heat exchanger. In this way, the exchanger would reduce its heat flow (J/s = W) from its maximum (when completely clean) to practically zero (when, due to scale, the primary or secondary side of the heat exchanger is completely impassable).

The device for chemical cleaning of the heat exchanger (Figure 2) serves for occasional cleaning of the primary or secondary side of the heat exchanger, without dismantling it from the network. The cleaning process takes about an hour. After isolation from the mains by closing the bay pass valve, cocktail of acids is injected into the primary or secondary side using an electromagneten pump. The pump pushes the acid that flows through the exchanger and thus dissolves the incrustations that have formed during its operation. After the process is completed, the acid is returned to the tank and the exchanger is rinsed with running water. After that, the bay pass valves are opened and the exchanger is operational again.

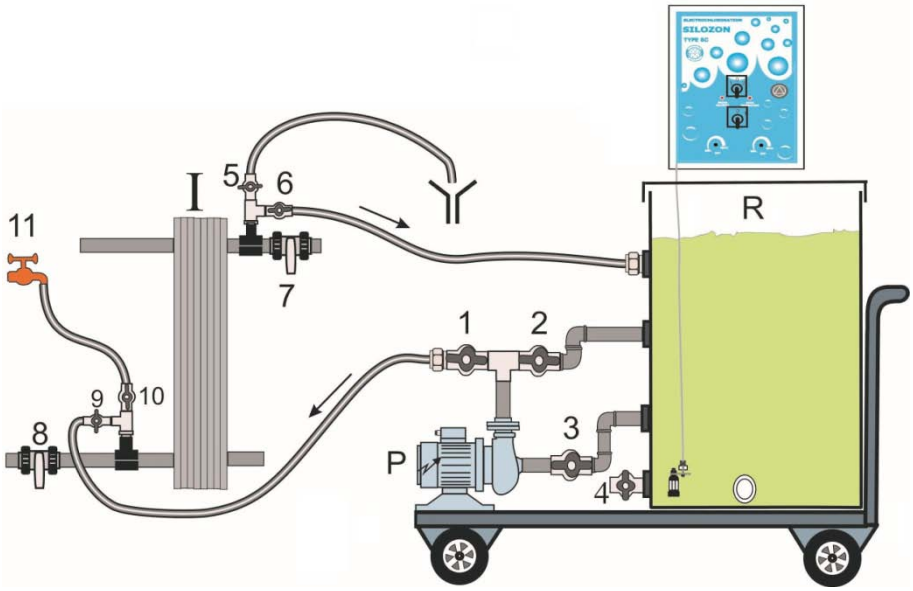

Figure 2. Device for chemical cleaning of heat exchangers

Technical data

The basic characteristics of the device are:
• Operating temperature: 10 - 38 °C;
• Operating voltage: 220 V AC;
• Capacity: 30 l/min;
• Power: 800 W;
• Maximum pressure: 1 bar.

Device installation requirements:
• Socket 220 V, 50 Hz, 1 kW;
• Connection ½", P = 0.5 - 3 bar;
• Connection to the sewage network min. Ø 50;
• Possibly a device that ensures the temperature in the room between 5 and 20 °C if the temperature in the room is lower than 5 or higher than 20 °C and the humidity is such that it does not reach the dew point so that the operational life of the electric components is longer;
• Given that there are large chemical vapors in the engine room, it is necessary to ensure air circulation through the engine room in order to ensure a longer life for all energy and electrical components and pumps;

• Lighting min bulbs 100 W;
• Ventilation drain Ø 70 for removing hydrogen and carbon dioxide without negative drops through the ventilation line;
• A dry room of 3 m^2 where the device is installed and at least 2 m^2 manupulative space around the device;
• That there is no condensation in the room for accommodation and operation of the device.

Commissioning

The device must be connected using a properly installed socket-outlet with a protected contact. When the electric plug of the device is installed in the AC outlet, the green LED will light up, indicating the presence of AC voltage at the input of the device. The tank is filled with acids from buckets of 10 or 20 l, and then tap water is added in the same amount as the acid was added. The amount of solution in the tank must be at least 10% greater than the volume of the primary or secondary side of the heat exchanger into which the solution is introduced.

Sequence of operations for device startup

First phase:
1. Connecting the valve assembly to the exchanger pipe;
2. Closing all valves;
3. Filling the vessel R with two primary volumes of the exchanger;
4. Opening the main valve on the pipeline;
5. Closing the bypass piping of the exchanger;

Second phase:
6. Opening valves 9, 5, 1, 2, 3;
7. Turning on the pump P;
8. Pouring one volume of primer into the bucket, turning off the pump;
9. Opening valve 6 and closing valve 5;
10. Turning on the pump P;

Third phase:
11. After 20 minutes, the pump is switched off;

12. Closing valve 9, opening valve 10 and faucet 11;
13. Filling the tank R to the initial volume, closing the tap 11;
14. Closing valve 6, opening valve 5 and faucet 11;
15. After 10 minutes, the tap is 11 closed;
16. Removing connections from valves 5, 6, 9, 10;
17. Armor penetration and opening of valves 7 and 8.

After use, the acid should be emptied from the tank and stored in canisters with caps that have a rubber seal.

Conclusion

The prototype of the device for chemical cleaning of heat exchangers was installed in the Tonanti Hotel in Vrnjačka Banja in the machine room where plate heat exchangers with power from 40 to 800 kW are located. All heat exchangers are plate type and are used to heat water in swimming pools and for central heating in rooms and common areas with the help of heat pumps. The heating fluid on the primary side of the heat exchanger is thermomineral water from arterial wells located near the hotel. Such water has high electrical conductivity, and is therefore rich in minerals - bicarbonates of calcium, iron and magnesium. The primary side of the 200 kW exchanger is heavily loaded with incrustations of calcium, magnesium carbonate, and iron hydroxide. After a few months of operation of the heat exchanger, a larger pressure drop was observed on the primary side of the exchanger when the cleaning process was initiated. In one hour and twenty minutes, the primary side was completely cleaned and the exchanger was then put back into operation. In a period of 18 months, the cleaning process was carried out several times, so that the device fully met expectations and proved its performance in exploitation on a real system - the machine plant of the Tonanti Hotel in Vrnjačka Banja.

Acknowledgement

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MOBILE DEVICE FOR WATER PURIFICATION WITH BOILER

Tomislav Trišović [1], Branimir Grgur [2], Zaga Trišović [3]

Abstract: Water is the best-known universal solvent, which in nature is almost always loaded with minerals. Those minerals can be removed by purifying water, which involves physical and chemical processes that produce higher-quality water.
The goal of this work was the development of an economical mobile device for softening and heating water used in packaging washing processes in the food industry. The designed device was built and assembled, and then tested in operation at the PIK Oplenac winery in Topola. It was concluded that the device provides the desired quality of packaging washing and the required amount of softened and heated water.

Keywords: water quality, ion exchange, water softening, washing, boiler.

Introduction

Food industry plants often require a higher quality of water than prescribed by the Rulebook for drinking water. For many elements present in drinking water, there is a maximum allowed concentration - MAC, as well as a maximum allowed concentration in waste water defined as emission limit value - ELV. Water hardness as a parameter has no MAC, so the user is left to judge for himself whether that parameter should be corrected in drinking water. Our water most often belongs to the group of hard and very hard water, so scale is deposited on elements of sanitary equipment and household appliances, and stains often appear on linen and dishes.

Water hardness can be of carbonate or non-carbonate origin. Transient or carbonate hardness originates from carbonates and bicarbonates of calcium and magnesium, when water is heated to boiling it is lost because the carbonate precipitate is translated into a precipitated solid state. Non-carbonate hardness -

[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia (trisa@tmf.bg.ac.rs)
[2]University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, 11000 Belgrade, Serbia (BNGrgur@tmf.bg.ac.rs)
[3] The Academy of Applied Technical Studies Belgrade, Katarine Ambrozić 3, 11000 Belgrade, Serbia (ztrisovic@atssb.edu.rs)

permanent hardness of water comes from sulfates, phosphates, chlorides, nitrates of calcium and magnesium.

Total hardness (TH) is equal to the sum of carbonate and non-carbonate hardness: TH = CH + NH, i.e. the sum of transitory and permanent hardness: TH = TH + PH, the SI unit of measuring water hardness is: mg $CaCO^3$ / dm^3 of water. In our country, hardness is expressed in German degrees.

Hardness can be reduced in several ways: thermally, magnetically and electromagnetically, high-frequency currents and chemically. In this article, chemical softening is applied.

Chemical softening of water is based on extracting salt, by adding chemical substances, from water in an undissolved form (sediment), which is removed from water by precipitation or filtration. Lime, soda, sodium hydroxide, phosphoric acid salts, etc. are used for chemical softening. The most commonly applied procedures are the decarbonization of water with lime, which is achieved with milk of lime:

$$Ca(HCO3)2 + Ca(OH)2 \rightarrow 2CaCO3\downarrow + 2H2O$$

$$Mg(HCO3)2 + Ca(OH)2 \rightarrow MgCO3\downarrow + CaCO3\downarrow + 2H2O$$

Water softening using the lime-soda process is achieved by removing salts insoluble in water after the action of lime (precipitation and filtration):

$$Ca(HCO_3)_2 + Ca(OH)_2 \rightarrow 2CaCO_3 + 2H_2O$$

$$MgCO_3 + Ca(OH)_2 \rightarrow Mg(OH_2) + CaCO_3$$

However, water-soluble salts remain in the water, which can be removed in the reaction with soda (chlorides, sulfates) by the following reactions:

$$CaSO_4 + Na2CO_3 \rightarrow CaCO_3\downarrow + Na_2SO_4$$

$$CaCl_2 + Na_2CO_3 \rightarrow CaCO_3\downarrow + 2NaCl$$

Today, one of the widely used ways of softening water is ion exchange. The following reaction takes place on the grains of the ion exchange material:

$$Ca^{2+} + 2Na - R = Ca - R_2 + 2Na$$

$$Mg^{2+} + 2Na - R = Mg - R_2 + 2Na$$

When the ion mass is saturated, it is necessary to regenerate it. The regeneration process is achieved using a saturated solution of table salt or acid for cationic masses, while anionic ion masses (anions are removed) are regenerated with sodium hydroxide according to the following scheme.

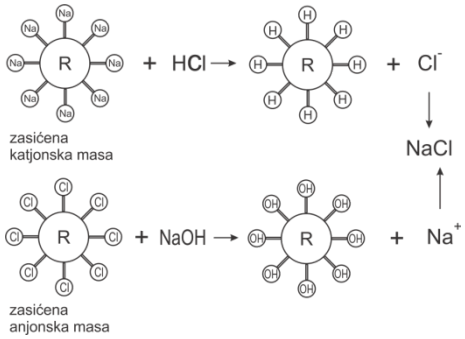

Figure 1. Scheme of cationic and anionic ion mass regeneration

Results and discussion

We designed and built a mobile device for softening and preheating water with a simple construction and low cost that can be used in small food industry plants where washing processes with hot and demineralized water are necessary. Such devices can also be used in other branches of the economy. The device has four components: a water softener, a boiler for heating water, a control cabinet and a mobile platform on which the equipment is fixed. Industrial wheels installed on the underside of the platform allow the device to be easily moved from one location to another along all production lines. For the operation of the device, it is necessary to supply it with alternating current 220V, 50Hz and a supply of drinking water.

The device works as follows. Inlet water of drinking water quality, with increased hardness, enters via valve V1 and candle filter Fs1 into the column with ionic mass KO, where transient water hardness (mainly bicarbonates of calcium and magnesium) is removed. When the water passed through the column, i.e. softened through the ionic mass, it exits through the filter candle Fs2. The production of softened water and the regeneration cycle are managed by an automatic valve head (AVG). The automatic valve head controls the operation of the water softener and determines the start of regeneration based on the measured volume of produced softened water (e.g. after 20 m^3), but in both cases the regeneration, which lasts about eighty minutes, is carried out at 2am (in the case of a single softener) when it is assumed that there is no need for softened water. Regeneration can also be started manually at any time.

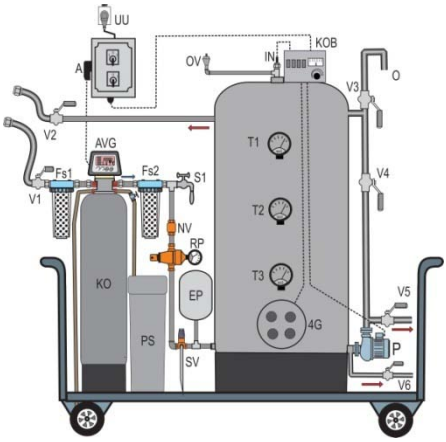

Figure 2. Drawing of a mobile device for softening and preheating water

The ionic mass column is usually made of composite material (polyethylene container reinforced with glass fibers and polyester). Inside the container, there is an ion exchange mass about 75% of the volume of the column. At the top of the column there is a nozzle for the entry of raw unsoftened water, and at the bottom a lower nozzle for the output of softened water (Figure 3).

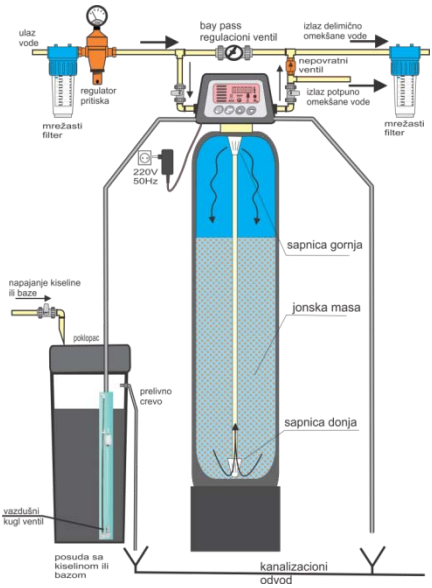

Figure 3. Single-column water softening unit with a brine

A container for making a saturated salt solution is called a brine (Figure 2). The volume of the brine is determined according to the volume of the ionic mass in the column. Tableted table salt is poured into the brine up to half of the container. There should be several centimeters of water above the salt tablet. At the bottom of the brine there is an inlet, ie. perforated bottom so that the water entering the container passing through salt tablets is saturated and as saturated (300 g/l) passes through the perforated bottom.

During the ionic mass regeneration process, a concentrated table salt solution is drawn from the bottom of the vessel with an ejector so that 120 to 200 g of table salt are consumed per liter of regenerated ionic mass. After that process, the same amount of fresh water is added to the brine so that the solution level in the brine is always the same. During the operation of the device, it is necessary to supplement the brine manually with salt tablets. There are five basic water softener cycles. The first cycle is normal operation when it produces softened water. Cycles from the second to the fifth cycle are types of regeneration.

The technological calculation of the capacity of the ion exchanger shows how much water can be softened in one working cycle, i.e. before it enters the regeneration process. If we have 75 l of ionic mass in the column and if the softening capacity is 4000 l/^{o}dH, then the total capacity of the softener is calculated: 4000 x 75 / 17 = 17600 l.

After the cumulative flow through the column of 17600 l of softened water, the regeneration process must be activated, which is achieved by programming the softener head. If the incoming water has a hardness of 17 ^{o}dH. We set the desired output water hardness on the water softener. All functions of the device (regeneration, rinsing) are regulated by the built-in automation.

The ion exchange water softener works fully automatically and is programmed according to the following parameters:

1. required flow of softened water of 500 liters per hour continuously during all 24 hours and
2. according to water hardness of 17 ^{o}dH (German degrees).

The condition for optimal operation of the softener is the optimal pressure in the pipeline for its supply, which is around 3 bar. The maximum pressure to which the column is certified is 10.5 bar. The automatic valve head works properly if the pressure is up to 6 bar.

The softener feeds the boiler with a volume of 500 l of water. The water heater has dry electric heaters and proper protection so that they cannot be

switched on if there is no water in the water heater. The boiler has a double set of thermostats that turn the heaters on and off to reach the optimum temperature. The water heater also has a recirculation pump that is turned on at the same time as the heaters are turned on in order to ensure a uniform, i.e. equal, temperature throughout the entire height of the water heater. Three thermometers are installed on the boiler at different heights in order to measure the temperature along the entire length of the boiler.

On the top of the boiler tank there is an IN level indicator that does not allow the boiler to start working until the water level reaches the top of the tank. If the water heater tank is not completely full, the water heating process will not start, so the signal lights on the control cabinet of the water heater KOB will not turn on. There is a thermostat button on the control cabinet of the KOB boiler, which is used to set the desired water temperature. After turning on the heater, the process of heating 500 liters of water lasts from 4 to 6 hours, depending on the temperature of the incoming water and the temperature of the environment. In the event of an increase in pressure over 6 bar, the safety valve SV opens and the pressure inside the vessel is relieved.

Conclusion

A prototype of a mobile water purification device with a boiler was installed in the PIK Oplenac winery in Topola in the bottling plant, where it is intended to soften and heat water for packaging washing. The device has been in operation for a long period of time and has met the expectations of investors. The device is periodically washed with an acid-based process and then disinfected so that, in periods when it is not working, algae and bacterial pollution do not occur. It is recommended that, after each wine filling and bottling season, the device is washed, disinfected, and the water heater completely emptied of water. The automatic valve head is programmed to regenerate and disinfect the column regardless of the fact that the water was not softened during that period. Since the device has proven to be reliable and provides quality water, we can recommend its use not only in wineries but also in other branches of the food industry and economy.

Acknowledgement

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PHENOLIC CONTENT AND *IN VITRO* ANTIOXIDANT ACTIVITY OF BLACK MULBERRY (*Morus Nigra* L.) FRUIT, JUICE AND POMACE

Nevena Matić[1], Nevena Barać[2], Danka Mitrović[1], Ivana Sredović Ignjatović[1], Miroljub Barać[1]

Abstract: Black mulberry fruits are a good source of valuable nutrients and a wide range of bioactive compounds including polyphenols, flavonols, polysaccharides and anthocyanins. To investigate the possible conversion of mulberry pomace as by-product into highly-valuable food additive, in this work the distribution of the total phenols, flavanols, monomeric and polymeric anthocyanins during black mulberry juice production was characterized. Also, the antioxidant properties (antioxidant capacity and ferric reducing power) of the obtained juice as well as water and methanol extracts of black mulberry pomace were detected. The results of this investigation clearly suggested that mulberry pomace is promising nutritional by-product for consindering in fuctional foods devolopment.

Keywords: black mulberry, phenols, pomace, antioxidant properties

Introduction

Mulberry is a fast growing deciduous plant found in wide variety of climatic, topographical and soil conditions, and is widely distributed from temperate to subtropical regions. It belongs to genus Morus as 24 species including *Morus alba* L. (white mulberry), *Morus nigra* L. (black mulberry) and *Morus rubra* L. (red mulberry) species.

Mulberry fruits are a good resource of carbohydrates, lipids, proteins, organic acids, vitamins, minerals, and fibers (Eyduran et al., 2015; Khalifa et al., 2018). Additionally, mulberry fruits contain a diversity of compounds (such as polyphenols, flavonols, polysaccharides and anthocyanins) that play a vital function in human metabolism (Akbulut and Özcan, 2009) and may have significant influence on human health. For example, according to Buhroo et al. (2018), consumption of black mulberry juice promotes healthy and smooth skin, prevents irritations, inflammations and throat infections, and also has laxative properties.

[1]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11 000 Belgrade, Serbia (isredovic@agrif.bg.ac.rs)

The level of bioactive compounds in different black mulberry products depends on several factors including their initial contents in mulberry fruits, processing conditions and storage conditions. Several researchers have studied the contents of phenolics (i.e., flavonoids and anthocyanins) and carotenoids in mulberry extracts and juices (Sánchez-Salcedo et al., 2015). However, according to our knowledge, the available literature lacks of data related to the distribution of these biologically active components during the production of black mulberry juice. In this work we characterized the distribution of total phenols, flavanols, monomeric and polymeric anthocyanins during black mulberry juice production as well as antioxidant properties of the obtained juice and pomace. The obtained data would indicate a possible conversion of the mulberry pomace into a highly valuable by-product.

Materials and methods

Samples and chemicals

The fruits of *Morus nigra* were collected from the Belgrade area in Serbia. The fruits were pressed on a hydraulic press to obtain the juice. Fruit, juice and pomace samples were store at -20°C until analysis.

All used chemicals were of analytical purity. Gallic acid, catechin, Folin-Ciocalteu phenol reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) from Sigma-Aldrich (Steinheim, Germany); CH_3OH, HCl, Na_2CO_3, NaOH, CH_3COONa, potassium bisulfite, $FeCl_3 \cdot 6H_2O$ from Merck (Darmstadt, Germany); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) from Fluka Chemie (Buchs, Switzerland) were acquired.

Extraction procedures

Three independent extractions for each sample were carried out using two extractants: water and 80% methanol solution (v/v). 3.0 ± 0.01 g and 2.5± 0.01 g of fruit and pomace, respectively, was extracted with 12.5 mL of water or methanol solution in a ultrasonic bath (Clifton, Series DU-14, North Somerset, USA) for 30 min. The treated samples were centrifuged (Yanetzki, Czech Republic) for 10 min at 4000 rpm and the supernatant was collected. Another 12.5 mL of extractant was added to the pellet and this extraction procedure was repeated two more times. All three supernatants were combined and adjusted to a final volume of 50 ml. Prepared extracts were stored at -20◦C until analysis.

Analytical procedures

The total phenolic content (TPC) was determined using Folin-Ciocalteu reagent as described by Natić et al. (2015). The absorbance at 760 nm was recorded using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). TPCs of black mulberries and pomace were expressed as mg gallic acid equivalents per 100 grams (mg GAE $100g^{-1}$) of the dry weight.

Total flavonoids were measured by a colorimetric assay desribed by Kim et al (2003). Total flavonoids of fruits and pomace were expressed on a dry weight basis as mg 100 g^{-1} catechin equivalents (CE).

DPPH assay was performed according to Chen et al. (2017.). DPPH radical scavenging ability of black mulberry fruit and pomace was expressed as μmol Trolox equivalent (TE) per 100 grams of dry weight sample.

The procedure of FRAP assay was adopted from the previous studies (Chen et al., 2017). The absorbance was determined at 593 nm and the results were expressed as μmol TE per 100 gram of dry weight of the sample.

The pH differential absorbance method was applied for total anthocyanins content determination using two different buffer systems (Lee et al., 2005).

Percent polymeric color was determined using the bisulfite bleaching method (Hager et al., 2008).

Results and discussion

Total phenolic content, antioxidant activity and flavonoids content

Results of spectrofotometric determination of TPC, DPPH, FRAP and TF using water and methanol (80%, v/v) extracts of black mulberry fruit and pomace are listed in Table 1. The results of our research are in agreement with the previously conducted analysis of black mulberry from Vojvodina (Natić et al., 2015). This observation applies to both aqueous and methanol extracts. However, based on the results shown in Table 1, it is clear that the TPC in the aqueous extract is twice as high as in the methanol extract. On the other hand, the DPPH antioxidant activity of the aqueous extract is slightly lower compared to that of black and white mulberry grown in Turkey (Eyduran et al., 2015; Özgen et al., 2009). It is evident that the DPPH activity of the methanolic extract is two times lower compared to the aqueous extract, while the results of the FRAP and TF analysis are comparable in both extracts.

Table 1. Total phenolic content, antioxdidant activity and total flavonoids

	TPC (mg GAE 100 g^{-1})	DPPH (μmol 100 g^{-1})	FRAP (μmol 100 g^{-1})	TF (mg 100 g^{-1})
BM-H	25.1±1.60	99.5±12.2	45.8±5.30	7.52±0.51
BM-M	12.7±1.70	49.8±6.36	41.0±3.13	6.51±0.62
BMP-H	6.17±0.18	26.3±1.93	3.50±0.60	1.72±0.13
BMP-M	6.06±1.58	28.3±2.30	84.8±8.72	2.61±0.23
	TPC (mg GAE L^{-1})	DPPH (mmol L^{-1})	FRAP (mmol L^{-1})	TF (mg L^{-1})
Juice	3050±160	14.1±1.20	8.97±1.14	797±50.2

Abervations: BM –black mulberry fruit. BMP – black mulberry pomace. H – water extracts. M – methanol extracts

Regarding mulberry pomace both extracts have similar values of total polyphenol content and DPPH antioxidant activity. A significant difference was observed for the FRAP assay. where the methanol extract had a significantly higher activity compared to the aqueous extract. Flavonoids are the second major mulberry polyphenols and presented mostly in glycosylated form, with complex flavonol glycosides profiles. The content of total flavonoids in pomace was 1.5 times higher in the methanolic extract.

Comparing with results of Khalid et all (2011) TP content in analysed black mulberry juice was higher than they reported. The average antioxidant activity of fresh juice according to a FRAP and a DPPH assay in our sample were slightly lower than reported in others investigations (Khalifa et al., 2018).

Total anthocyanins content and polymeric color

Anthocyanins are the predominant polyphenols in mulberry fruits, and mulberry fruits have higher anthocyanins content than blueberry, blackberry, blackcurrant, and redcurrant (Veberic et al., 2015). Methanol solution was better extractant for anthocyanins from fruit and pomace samples (Table 2). When compare, it is evident that TAC in analysed fruit sample was five times lower than reportet by other authors (Ozgen, 2009; Sánchez-Salcedo et al., 2015). The juice sample had higher TA content comparing to cranberry and strawberry juices, but lower than raspberry juice (Lee et al., 2005).

One of the most important bioactive constituents of mulberries is the anthocyanins, mainly responsible for the color of mulberry fruits (60%). These water-soluble pigments have dual value: first, they constitute an integral part of the sensory attributes since their levels and various forms pertain directly to the coloration of the final product; and second, they are thought to possess diverse

biological properties and therefore are considered as secondary metabolites with potential nutritional value (Khalifa et al., 2018). The percent polymeric color determined in water and methanol extracts for fruit sample were the same. But pomace extraction showed smaller percent in methanol than water extract. When compared to black raspberry (Hager et al., 2008), black mulberry juice has significantlly higher percent of polymeric color.

Table 2. Total anthocyanin content and polymeric color

	BM-H	BM-M	BMP-H	BMP-M	Juice	
TAC (mg C3G 100 g^{-1})	45.0±8.05	280±45.7	23.3±3.51	150±43.2	TAC (mg L^{-1})	155±10.8
PC, %	77.7±0.62	77.7±7.34	83.2±2.15	72.8±1.26	PC, %	73.6±3.46

Abervations: BM –black mulberry fruit. BMP – black mulberry pomace. H – water extracts. M – methanol extracts

Conclusion

In this study, characterization and comparison of the phytonutrient content and antioxidant capacity of two different extracts were performed. The obtained results indicated that for investigated quality parameter water was better extractant for black mulberry fruits, but for pomace it was methanol solution. The novelty of the presented research lies in the analysis of black mulberry pomace, and results suggested it is promising nutritional by-product for consindering in fuctional foods devolopment.

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BOVOLID – CHEMICAL COMPOUND FOR EVALUATING THE AGE OF WHITE MULBERRY LEAVES

Vojkan Miljković[1], *Marko Mladenović*[2], *Niko Radulović*[2]

Abstract: Tree *Morus* species are growing in Serbia, *Morus alba, Morus nigra* and *Morus rubra. Morus* leaves are used in traditional medicine, and after collecting them, drying is most practiced method for preservation. Hydrodistillation of 2 fresh and 2 dryed for one month samples of *Morus alba* and *nigra*leaves was done by using the original Clevenger-type apparatus. The composition of essential oils was determined by GC-MS aparathus. The yields of essential oils obtained by hydrodistillation were 0.0011 and 0.0012% from the dried plant material (MNL-2 and MAL-2, respectively) and 0.0080% (w/w) from both samples of fresh leaves (MNL-1 and MAL-1). The predominant constituent in all essential oils was trans-phytol. Only the essential oil obtained from dried *M. alba* leaves contained the chemical compound bovolid (8.1%). Based on results, bovolid can be used as indicator for evaluating the age of white mulberry leaves.

Keywords: *Morus alba*, *Morus nigra*, essential oil, bovolid

Introduction

The genus *Morus* L. (Moraceae) consists of about 16 species that are wild growing or cultivated trees. They are regularly called mulberries and their fruits taste delicious (Josifović, 1975). They have been used for a long time in folk medicine and have proven medical activities (Miljković et al., 2014). Tree *Morus* species grow in Serbia, *M. alba* (white mulberry), *M. nigra* (black mulberry) and *M. rubra* red mulberry. Interestingly, in archives dating from 13th century, one of the oldest tree in Serbia is black mulberry tree in the Patriarchate of Peć (Josifović, 1975). In commercial production of silk, white mulberry is cultivated for feeding silkworms with it's leaves (Josifović, 1975) because of high protein and amino acids content (Machii, 1989). According to literature, fresh or dried mulberry leaves can be used for medicinal purposes

[1]University of Niš, Faculty of Technology, Bulevar oslobođenja 124, Leskovac, Serbia (vojkan@tf.ni.ac.rs)

[2]University of Niš, Faculty of Sciences and Mathematics, Department of Chemistry, Višegradska 33, Niš, Serbia

(Bown, 1995). Mulberry leaves, like other herbal teas or herbs used medicinally, are often used as a dry drug for storage or consumption purposes.

The most practiced method for post-harvest preservation of medical plants is drying (Muller & Heindl, 2006). To prevent growth of microorganisms and to make storage and transportation of herbal material easier the drying process of plant material is practiced. Throughout the drying process, evaporation, oxidation by atmospheric oxygen, degradation and transformation of some chemical compounds occurs. There are published work reporting organoleptic as well as changes in chemical composition on the constitution of essential oil as a result of drying of plant material (Blagojević et al., 2015).

Bovolide has been isolated from sources such as: butterfat (Boldingh & Taylor, 1962), tobacco (Demole & Berthet, 1972), peppermint oil (Takahashi et al., 1980). It was confirmed previously that bovolide and dihydrobovolide are formed during biochemical transformations after harvesting plant material under the influence of light from initial plant metabolites, which usually follow the process of plant material (Horita et al., 1985). Both bovolide and dihydrobovolide have a strong aroma, similar to celery, and are patented products used to improve the taste of tobacco (Boidingh & Taylor, 1962; Lardelli et al., 1966).

Exposure of green tea leaves to light resulted in formation of a flavor after period of time. Chemical analysis showed that content of dihydrobovolide increased after this. Also, in dry leaves of species *Gardenia jasminoide*s L. and *Magnolia grandflora* L. the content of bovolide increased from 0.22 and 0.56 to 3.08 and 2.19 respectively [Horita et al., 1985].

In order to give description of *Morus alba* L. and *Morus nigra* L. leaves chemical composition, analysis of the essential oil from both fresh and dried leaves of two *Morus* species (*M. alba* and *M. nigra*) cultivated in Serbia was performed. The aim of this work is to propose the possibility of differentiating the age of dried mulberry leaves based on the presence of the chemical compound bovolide.

Materials and methods

Plant material: *Morus nigra* and *Morus alba* leaves collected in July, 2015 (village Gornji Barbeš, near Gadžin Han, Serbia), were identified and voucher specimens (VM0012 and VM0013) were deposited in the Herbarium of the Faculty of Science and Mathematics, University of Niš, Serbia. A portion of the leaves was subjected to hydrodistillation immediately after collection, while another portion was dried for 1 month at room temperature, without direct

exposure to sunlight. Samples were labeled: MAL-1 (fresh *Morus alba* leaves), MAL-2 (dryed *Morus alba* leaves), MNL-1 (fresh *Morus nigra* leaves) and MNL-2 (dryed *Morus nigra* leaves).

Essential oils obtaining: Fresh (3 × 300 g batches) and dried leaves (3 × 100 g batches) were hydrodistillated for 4.5 h using the original Clevenger-type apparatus (Blagojević et al., 2015).

GC-MS analysis: GC-MS analyses (3 repetitions) were performed by using a Hewlett-Packard 6890N gas chromatograph equipped with a fused silica capillary column DB-5MS (5% phenylmethylsiloxane, 30 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies, USA) and coupled with a 5975B mass selective detector manufactured by same company. The injector and interface were operated at 250 and 300°C, respectively. Oven temperature was raised from 70 to 290°C at a heating rate of 5°C/min and the program ended with an isothermal period of 10 min. As a carrier gas helium at 1.0 mL/min was used. The samples, 1.0 μL of essential oil solutions in diethyl ether (10.0 mg of an essential oil sample per 1.0 mL of solvent), were injected in a pulsed split mode (the flow was 1.5 mL/min for the first 0.5 min and then set to 1.0 mL/min throughout the remainder of the analysis; split ratio 40 : 1). MS conditions were as follows: ionization voltage 70 eV, acquisition mass range m/z 35-650, scan time 0.32 s. Percentage composition of the essential oils was calculated from the GC peak areas without any corrections. Constituents were identified by comparison of their linear retention indices (relative to C8-C33 n-alkanes on a DB-5MS column) with literature values (Adams, 2007) and their mass spectra with those of authentic standards, as well as those from Wiley 6, NIST11, MassFinder 2.3, and a homemade MS library with the spectra corresponding to pure substances and components of known oils, and wherever possible, by co-injection with an authentic sample.

Results and discussion

The yields of essential oils obtained by hydrodistillation were 0.0011 and 0.0012% from the dried plant material (MNL-2 and MAL-2, respectively) and 0.0080% (w/w) from both samples of fresh leaves (MNL-1 and MAL-1). The most represented compounds classes were alkanes and diterpenoids which together constituted more than 60% of essential oil obtained from dried *M. alba* leaves, and even more than 88% of essential oil obtained from fresh and dried *M. nigra* leaves, and fresh *M. alba* leaves. Also, the essential oils were consisted of the carotenoids in range of 1.4-24.8% and fatty acid related constituents were

up to 8.1%. The main constituents of essential oils obtained from *M. nigra* leaves were: trans-phytol (65.4-71.2%), (E,E)-geranyl linalool (0.2-8%) and normal chain alkanes (tricosane 2-2.8%, pentacosane 2.4-3.8%, heptacosane 1.9-2.7%, nonacosane 3.4-4.1% and hentriacontane 2.4-3.5%. Similar constitution was found to be in both *M. alba* essential oils, with alkanes, diterpenoids and carotenoid derivatives. The predominant constituent was trans-phytol (7.9-61.6%).

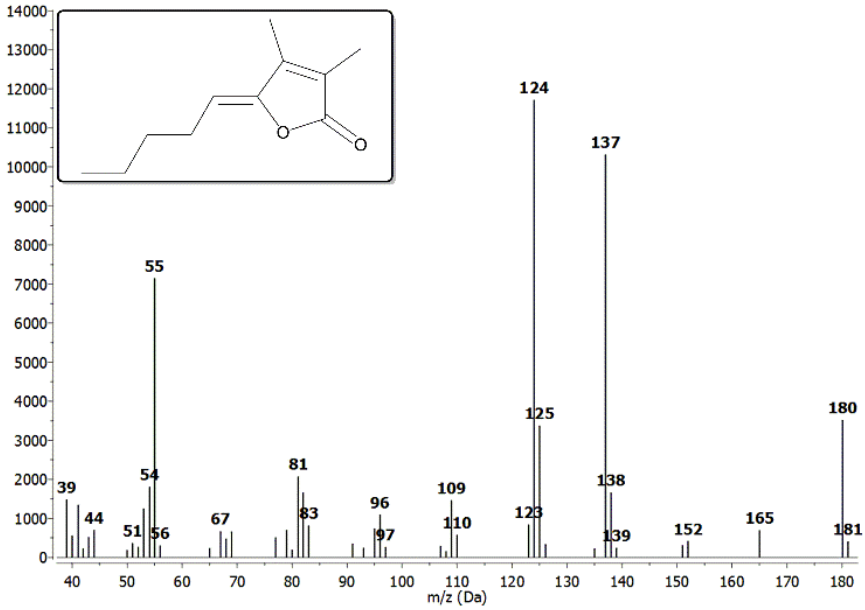

Figure 1. Mass spectrum and structure of cis-bovolide ((Z)-3,4-dimethyl-5-pentylidene-2(5H)-furanone)

The essential oil obtained from dried *M. alba* leaves in it's composition had a chemical compound with retention index (RI) 1518, Figure 1. It was present in essential oil with 8.1%. Interestingly it wasn't constituent of essential oil obtained from fresh *M. alba* leaves. A first attempt to identify this compound with molecular ion at m/z 180 and base peak at m/z 124 by comparison of the mass spectrum with ones from the mass spectral libraries like Adams, Wiley 6, NIST11 and MassFinder 2.3 did not gave a result. From the author's experience this compound was already known as a constituent of numerous others essential oils (Radulović et al., 2012). Initial step to identification was a dyhidrobovolide, a constituent eluting just before compound of interest at

RI=1512. Ions of dyhidrobovolide with molecular ion at m/z 182 were 2 amu higher in mass spectrum than ones of still unidentified compound. Then, it was considered that unidentified compound at RI=1518 is actually a dehydrogenated derivative of dihydrobovolide, and it was found in literature that (E/Z)-3,4-dimethyl-5-pentylidene-2(5H)-furanone known as (cis/trans)-bovolide exists. By comparison of reported mass spectrum (MacLeod, 1991) and RI value obtained under same conditions (column of the same polarity) the identity of bovolide was confirmed.

Conclusion

Essential oils of fresh and dried *Morus alba* L. and *Morus nigra* L. leaves were obtained by hydrodistillation and their qualitative and quantitative analysis was done using GC-MS technique. New chemical compound – bovolide was identified and its mass spectrum is given. It was present only is dried *Morus alba* L. leaves. Based on the results of chemical analysis it can be concluded that presence of bovolide in dried *Morus alba* L. leaves is indicator for leaf age. Please check each item to be certain that the article is in compliance with the requirements.

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THE DIFFERENCE IN LYCOPENE AND β-CAROTENE CONTENT IN *CITRUS PARADISI* FRUIT AND *ROSA CANINA* DRIED FRUIT

Vojkan Miljković[1], Ivana Gajić[1], Jelena Mrmošanin[2], Milica Nešić[2]

Abstract: *Citrus paradisi* fruit and *Rosa canina* dried fruit are rich sources of vitamin C. The aim of this work was to highlight their content of lycopene and β-carotene in order to contribute to their chemical profile. For that purpose the conventional extraction method was applied with solvent mixture consisting of 250 ml hexane, 125 ml of acetone, 125 ml of ethanol (2:1:1, v/v/v), and 0.05 % (w/v) butylated hydroxytoluene (BHT). UV-Vis spectrophotometric method was performed and for final calculation of concentrations was used Lambert-Beer's law. Obtained results for content of lycopene and β-carotene in *Citrus paradisi* fruit and *Rosa canina* dried fruit are 2.27 and 2.34, that is 2.28 and 7.25 mg per 100 g/fruit weight (FW), respectively. The obtained results are recommending these two nutrients in everyday diet.

Keywords: *Citrus paradisi, Rosa* canina, lycopene, β-carotene first

Introduction

In Asia, North America, Middle East and Europe are growing more than 100 species of *Rosa* genus (Nilsson, 1997). Beside the name rose hip, *Rosa canina* L. is also known by names: dog rose, dog brier, wild briar, hip fruit, hip tree (Tutin et al., 1992). It's fruit (cynosbati fructus) is known as valuable source of vitamin C (Fan et al., 2014). Moreover, rose hips have highest vitamin C content (up to 1300 mg/100 g of fruit) amid fruits and vegetables (Chai & Ding, 1995). The experimental results performed in order to give comparison of vitamin C content in *Rosa canina* L. fruit and one citrus fruit showed that rosehip is richer source (Roman et al., 2013).

Citrus fruits are one of the most represented fruits consumed fresh in everyday diet. Alongside the vitamic C they are source of flavonoid compounds that are contributing to health benefits (Shi et al., 2020). Not the first, but the

[1]University of Niš, Faculty of Technology, Bulevar oslobođenja 124, Leskovac, Serbia (vojkan@tf.ni.ac.rs)

[2]University of Niš, Faculty of Sciences and Mathematics, Department of Chemistry, Višegradska 33, Niš, Serbia

second largest produced citrus on global is grape, with average production of 60 million tons per annum (Schieber et al., 2001). *Citrus paradisi* (red grapefruit) is a member of *Citrus* genus with mild sour to semi sweet taste. It has wide spectrum of applications as food source, in cosmetics and folk medicine. Okunowo et al. discovered that some compounds in essential oil of *Citrus paradisi* peels contain antibiotic principles [Okunowo et al., 2013].

β-carotene is natural carotenoid that can be found as constituent of plants and fruits. It is yellow-orange colored substance with antioxidant properties and health promoting effect (Daood et al., 2014). In human body carotenoids are getting converted into vitamin A. Concretely, β-carotene reduces chances for cataract, coronary disease, some types of cancers. It has immunostimulant effect. For plants, carotene synthesis is important for their growth, photosynthesis and for synthesis of phytohormones (Miljković et al., 2021).

Lycopene is a natural red-orange carotenoid. It can be found in the group of the strongest natural antioxidants, as constituent of tomatoes, grapefruits, red oranges, etc. Efficiency of lycopene is shown against degenerative diseases such as cancer of the lungs, bladder, cervix, atherosclerosis and associated coronary artery disease, prostate (Harini & Judia Harriet Sumathy, 2016). By consuming 6 mg per day chances for getting prostate cancer are reduced by 10-20%. Therefore, it's presence in daily diet is of importance.

The aim of this work is to determine lycopene and β-carotene content in fresh fruit of *Citrus paradisi* and *Rosa canina* dried fruit.

Materials and methods

Plant material: Mature grape fruits was purchased in March of 2022 at the local market in Niš, Serbia. Fruits were washed with deionized water, sliced and homogenized in Brown® blender. Fresh *cynosbati fructus* was bought in March of 2022 at the local market in Niš, Serbia. The fruits were washed, and left drying in the shade. After that, dried fruits were sliced and homogenized in Brown® blender.

Chemicals: Acetone was purchased from Fisher Scientific (Loughborough, United Kingdom). Hexane, butylated hydroxytoluene, sodium acetate, ethanol and glacial acetic acid were purchased from Sigma-Aldrich (Steinheim, Germany).

Spectrophotometric determination of lycopene and β-carotene content: The conventional extraction method was applied in this experimental research (Perkins-Veazie et al., 2001; Sadler et al., 1990). Precisely, 10 g of each sample (dried rosehip fruit – sample 1 and red grapefruit – sample 2) were mixed with a solution consisting of 250 ml hexane, 125 ml of acetone, 125 ml of ethanol (2:1:1, v/v/v), and

0.05 % (w/v) butylated hydroxytoluene (BHT). The stoppered mixture was placed on an orbital shaker to mix at 180 rpm for 150 minutes (temperature of mixing was 5 °C). After mixing, 75 ml of cold deionized water was added and the mixture was agitated for another 5 min. In order to allow the separation of polar and non-polar layers, the suspension was left at room temperature for 10 minutes. The extract was re-dissolved in hexane. Then, the hexane extracts were scanned in the visible light wavelength range of 400-750 nm using Jenway 6105 UV/Vis spectrophotometer (Jenway, United Kingdom) in 1 cm path length quartz cuvette blanked with n-hexane and the maximum absorbance were observed at 450, 472 and 503 nm, respectively for the lycopene/β-carotene hexane layer mixture versus a blank of the above-mentioned solvent mixture. The molar extinction coefficient of 172 000 L mol-1 cm-1 at 503 nm was used for quantification, applying the Beer-Lambert law (Ravelo-Perez et al., 2008; Zechmeister et al., 1943).

Results and discussion

In Figure 1 is shown typical overlap spectra of β-carotene and lycopene with absorption maxima at 450 nm, 472 nm and 503 nm. This is because most carotenoids show absorbance maxima at three wavelengths in a three peak spectrum. And, with the increase of conjugated double bonds come λmax shifts to longer wavelengths. In that way lycopene, the most unsaturated acyclic carotenoid, with 11 conjugated double bonds is red and absorbs at the longest wavelengths (λmax at 443, 471, 503 nm) (Rodriguez-Amaya and Kimura, 2004).

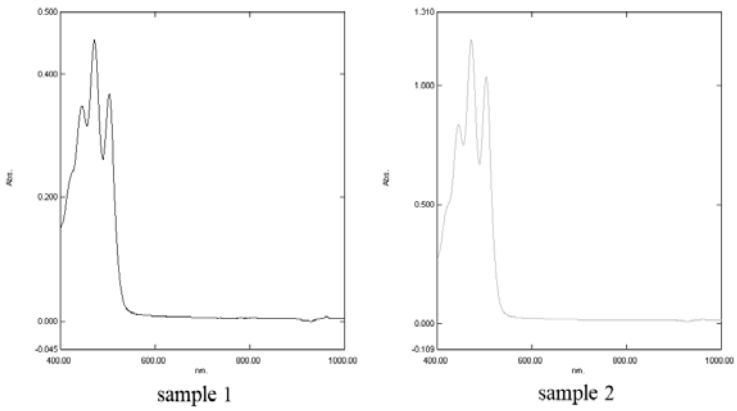

Figure 1. Absorption spectra of the β-carotene-lycopene mixture in hexane extract of *Citrus paradisi* (1) and *Rosa canina* (2)

β-carotene, also in that way, with the same number of conjugated double bounds is yellow-orange and shows absorption peaks at 450 and 472 nm and a mere shoulder at 425 nm (Rodriguez-Amaya and Kimura, 2004). This is because both quantified carotenoids absorb in the overlapping wavelength ranges. Nevertheless the fact that wavelength value 503 nm is not absorption maximum makes it acceptable [Ravelo-Perez et al., 2008].

By following the law of Lambert and Beer, the absorbance at 450 nm and 503 nm (in a quartz cuvette 1 cm wide) of the carotenoid mixture of lycopene and β-carotene was measured and the final results are shown in Table 1.

Table 1. β-carotene and lycopene content of *Citrus paradisi* fruit and *Rosa canina* dried fruit in n-hexane extracts

Compound	Sample	
	Citrus paradis fruit	*Rosa canina* dried fruit
β-carotene (mg/100 g FW)	2.28 ± 0.07	7.25 ± 0.09
Lycopene (mg/100 g FW)	2.27 ± 0.02	2.34 ± 0.06

Conclusion

Determination of β-carotene and lycopene in Citrus paradisi fruit and Rosa canina dryed fruit was done. By comparison of obtained results it can be concluded that both nutrients are similar in lycopene content, but when it comes to β-carotene Rosa canina dried fruit contains more than 3 times higher concentration of this carotenoid. In addition to the fact that they are rich sources of vitamin C, obtained results are recommending them even more to be consumed in everyday diet. Spectrophotometrical method is an appropriate method for β-carotene and lycopene determination with accuracy in results. It would be interesting to compare the content of determined substances in processed food products made of them.

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CLASSIFICATION OF FRUIT TREE LEAVES ACORDING TO PHENOLIC PROFILE USING PRINCIPAL COMPONENT ANALYSIS

Milan Mitić[1], Jelena Mitić[1], Jelena Nikolić[1], Pavle Mašković[2]

Abstract: This study aimed to determine and compare a polyphenolic profile of the fruit tree leaves of ten commonly commercially and wildly consumed fruits: apricot, plum, peach, quince, apple, blackcurrant, redcurrant, blackberry, raspberry, and dog rose. The polyphenolic profile was determined using HPLCmethod.Inleaves were identified 17 individual phenolics. To evaluate relationships through various fruit tree leaves, Principal Component Analysis (PCA)was employed. Two statistically significant components were identified, grouping fruit tree leaves based on phenolic compounds content.

Keywords: tree leaves, polyphenolic profile, HPLC, Principal component analysis

Introduction

In recent years, several reports have been published on the use of anatomical parts of these plants other than fruits as a source of active phytochemicals (Ferlemi and Lamari, 2016). The phenolic profile of plant tissues ate known to be affected by many factors such as genotype, environment, growth stage, time of harvesting, process and storage conditions and method of analysis (Pavlović et al., 2019). Studies on the potential of leaves, which are plant waste, demonstrate their usage by "green chemistry" principles.

Large datasets are increasingly widespread in many disciplines. To interpret such datasets, methods are required to drastically reduce their dimensionality in an interpretable way, such that most of the information in the data is preserved. Many techniques have been developed for this purpose, but PCA is one of the oldest and most widely used. Its idea is simple—reduce the

[1]University of Niš, Faculty of Science and Mathematics, Višegradska 33, Niš, Serbia (milan.mitic1@pmf.edu.rs)
[2]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia

dimensionality of a dataset, while preserving as much 'variability' (i.e. statistical information) as possible (Ian T.Jolliffeand Jorge Cadima, 2016).

In the current study, we investigated the major flavonol glycosides and phenolic acids in the leaves of two of the most important commercial *Grossulariaceae* varieties and eight *Rosaceae* varieties in Serbia, using high-performance liquid chromatography (HPLC) combined with a diode array detection system (HPLC-DAD), as well as their classification using the principal component analysis.

Materials and methods

Plant materials

Ten fruit tree leaves were obtained samples from local growers (Nis, Serbia). All samples were harvested at the same time-2 weeks after full flowering. Immediately after the harvest leaves, were air-dried in shade and ground.

Extract preparation

About 1g of the grounded leaf samples were extracted three times using methanol (purity 99.8%) using an ultrasonic bath for 15 min. The resulting extracts were filtered through Whatman filter paper (black ribbon) and dissolved with methanol to the final volume of 25 mL.

HPLC analysis

Qualitative analysis of phenolic acids, flavonols, and flavonols was performed with the HPLC, employing a direct injection method (Mitić et al., 2012).

Results and discussion

The contents of individual phenolic compounds (mg/g d.w.) of fruit leaves are shown in Table 1 and Table 2. Eight flavonols (quercetin-3-rutinoside, quercetin-3-galactoside, qurcetin-3-glucodide, qurcetin-3-rahmoside, kamferol-3-rutinoside, kamferol-3-glucoside, quercetin and kamferol), two flavonons (luteolin-7-glycoside and apigenin-7-glycoside) and 7 phenolic acids (3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, 3,5-*O*-dicaffeoylquinic acid; caffeic acid, p-coumaric acid and elagic acid) were

identified and quantified. The results indicate significant differences ($p < 0.05$) in flavonoids between the analyzed species.

Table 1. Polyphenols content and profile in leaves of tested plant species (mg/g d.w.)

Sample	Quince	Apricot	Apple	EU Plum	Peach
3-CQA	0.923±0.063	1.056±0.034	-	0.234±0.021	-
4-CQA	0.018±0.003	0.235±0.005	-	0.153±0.011	-
5-CQA	2.922±0.140	3.032±0.063	1.022±0.085	2.055±0.111	1.532±0.050
3,5-diCQA	0.655±0.045	-	-	-	-
CA	-	0.054±0.008	0.322±0.022	0.140±0.009	0.308±0.014
p-CA	0.010±0.001	0.032±0.001	0.586±0.032	-	0.146±0.005
EA					
Q-3-Rut	0.955±0.054	0.173±0.007	3.736±0.185	1.365±0.104	0.935±0.055
Q-3.Gal	0.909±0.036	1.299±0.025	0.458±0.092	0.280±0.021	-
Q-3-Glu	0.599±0.028	0.468±0.018	-	0.726±0.034	-
Q-3-Rhm	-	-	0.712±0.066	0.105±0.007	-
K-3-Rut	0.306±0.022	0.203±0.015	-	-	0.405±0.019
K-3-Glu	0.197±0.008	-	0.232±0.017	-	1.867±0.047
Q	0.064±0.002	-	0.150±0.008	0.205±0.050	0.682±0.031
K	-	-	-	0.065±0.003	1.015±0.074
L-7-gly	-		0.205	-	
A-7-gly	-		0.092	-	

Table 2. Polyphenols content and profile in leaves of tested plant species (mg/g d.w.)

Sample	Blackcurrant	Redcurrant	Blackberry	Raspberry	Dog rose
3-CQA	0.465±0.017	0.443±0.012	-		0.287±0.009
4-CQA	-	-	-		0.105±0.003
5-CQA	2.234±0.020	1.125±0.029	0.278±0.010	0.263±0.009	0.943±0.096
3,5-diCQA	-	-	-	-	-
CA	0.206±0.015	1.086±0.017	0.432±0.024	0.782±0.053	0.126±0.004
p-CA	0.297±0.011	0.107±0.005	1.020±0.065	0.936±0.031	0.045±0.002
EA			3.012±0.082	2.922±0.154	3.054±0.098
Q-3-Rut	2.227±0.014	3.872±0.206	0.381±0.015	0.952±0.075	0.735±0.022
Q-3.Gal	1.123±0.052	2.162±0.183	-	0.245±0.026	0.958±0.028
Q-3-Glu	4.322±0.266	1.622±0.018	12.096±0.956	4.083±0.121	1.345±0.019
Q-3-Rhm	-	-	-	-	1.985±0.032

K-3-Rut	0.123±0.008	0.755±0.016	0.636±0.028	0.032±0.005	0.865±0.018
K-3-Glu	0.237±0.008	0.410±0.009	-	1.118±0.053	1.866±0.026
Q	0.165±0.005	0.299±0.011	-	0.211±0.006	
K	-	0.028±0.003	-		
L-7-gly	-	-	0.182±0.013	0.389±0.033	0.353±0.017
A-7-gly	-	-	0.012±0.002	-	

The content of phenolic compounds in 10 fruit tree leaves samples were subjected to PCA analysis to better understand the relationship between analyzed samples (Figure1). PCA was applied to standardized data, so each parameter contributes equally to the data set variance and carries equal weight in the principal component calculation.

Six principal components were obtained (PC1, PC2, PC3, PC4, PC5 and PC6) accounted for 30.75%, 18.72%, 15.14%, 13.31%, 9.29% and 6.66% of the total variance, respectively. This statistical analysis resulted in a six-component model that explains 89.161% of the total variance between the results obtained.

L-7-gly (0.798), EA (0.785), p-CA (0.772), CA (0.530), Q-3-Rhm (0.317), K-3-Glu (0.289), Q-3-Glu (0.552), K-3-Rut (0.289) and A-7-gly (0.366) are strongly positive correlated and grouped on the plot (Fig. 1). In contrast, 5-CQA (-0.985), 3-CQA (-0.823), Q-3-Gal (-0.402), 3.5di-CQA (-0.470) and 4-CQA (-0.519) are on the negative side of the plot.

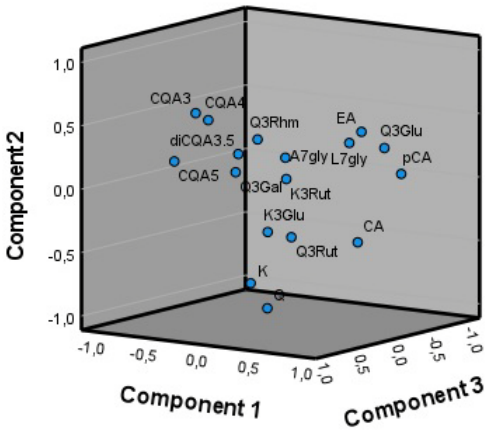

Figure 1. Plot obtained by principal component analysis

The first principal component distinctly separates wild blackberry (1.31) and wild raspberry leaves (1.32) from all other fruit tree leaf samples, because

they have the highest positive loadings on PC1.The second principal component distinctly separates dog rose leaves from all others because dog rose leaves (1.43) have the highest positive loadings on PC2.

The other hand, The Q-3-Rhm (0.76) and K-3-Glu (0.76), have the highest positive loadings on PC3 and contribute the most to the third component.PC4 was loaded by positively correlated redcurrant and apple leaves (1.2 and 2.0) and negatively correlated peach leaves (-1.4) and explained 13.31 % of the total variance.PC5 component explained 9.29 % of the total variance, strongly influenced by the positively correlated variable, redcurrant leaves (1.5). PC6 explained 6.66 % of the total variance, and it is made up of apricot (-1.4) and quince leaves (2.3).

Conclusion

The results demonstrate that fruit tree leaves are promising sources of phenolic compounds, especially wild tree leaf species. Principal component analysis grouped analyzed fruit tree leaf species regarding their phenolic composition. The study, therefore, presents data that attest to the importance of fruit tree leaves in providing the much-needed dietary important sources of phenolic compounds. Fruit tree leaves are a significant and rich source of these compounds.

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KINETICS OF THE REACTION OF AN ARYLIDENE 2-THIOHYDANTOIN DERIVATIVE WITH SOME Pd(II) COMPLEXES

Petar Stanić[1,], Darko Ašanin[1], Marijana Vasić[2], Tanja Soldatović[3], Biljana Šmit[1]*

Abstract: Reactions of 3-(benzylideneamino)-2-thioxoimidazolidin-4-one with some palladium complexes ($PdCl_2$, $Pd(DMSO)_2Cl_2$ and K_2PdCl_4) were monitored with NMR spectroscopy, which is used as a convenient and practical tool for determining the kinetic parameters of the reactions. Rate constants of the reactions were determined and reactivity of the complexes towards the 2-thiohydantoin derivative was compared.

Keywords: kinetics, 2-thiohydantoin, NMR, complexes, Pd(II)

Introduction

Thiohydantoins are sulphur analogues of hydantoin where at least one of the carbonyl groups is replaced with a thiocarbonyl group (Metwally and Abdel-Latif, 2012). The most recognizable and certainly the most researched compounds out of this group are 2-thiohydantoins. They are a valuable scaffold, with many biological, pharmacological and agriculutral activities, with applications ranging from medicine and therapy to industry (Gawas et al., 2021). They possess a plethora of biological activities, such as antimicrobial (antibacterial and antifungal) (Marton et al., 1993), anti-HIV (Khodair et al., 1997), antimutagenic (Takahashi et al., 1996), anticancer (Al-Obaid et al., 1996), anticonvulsive (Habib et al., 2015), anti-ulcer and anti-inflammatory (Curran, 1976). Moreover, 2-thiohydantoins are used as reference standards for C-terminal protein sequencing (Mo et al., 1997), reagents for textile printing dye development (Nelson et al., 1997) and are used as well for metal complexation and polymerization catalysis (Kandil et al., 2004).

[1]University of Kragujevac, Institute for Information Technologies, Jovana Cvijića bb, Kragujevac, Serbia (petar.stanic@uni.kg.ac.rs)

[2]Academy of Professional Studies Šumadija, Department in Kruševac, Kosančićeva 36, Kruševac, Serbia

[3]State University of Novi Pazar, Department of Chemical Technological Sciences, Vuka Karadžića bb, Novi Pazar, Serbia

2-Thiohydantoins possess a considerable coordination potential. Despite the small size of the 2-thiohydantoin molecule, it has four derivatization sites, which make its derivatives versatile ligands. Apart from the heteroatoms in the ring, the derivatives for the most part have heteroatoms in the side branches suitable for coordination and complexation. Many 2-thiohydantoin complexes have been synthesized so far. Particularly, arylidene 2-thiohydantoin complexes have been studied extensively, mostly because of their biological properties, such as antimicrobial and anticancer (Ismail et al., 2022).

In this study, for the purpose of examining the coordination potential of an arylidene 2-thiohydantoin derivative, 3-(benzylideneamino)-2-thioxoimidazolidin-4-one, towards palladium, the compound was subjected to ^{1}H NMR monotiring of its reactions with some Pd(II) complexes, $PdCl_2$, $Pd(DMSO)_2Cl_2$ and K_2PdCl_4. Studying the kinetics of the reaction will prove useful for the elucidation of mechanisms of 2-thiohydantoin coordination.

Materials and methods

All chemicals and reagents used in this study were commercially available and of high purity and were used in their obtained state, without further purification. 3-(Benzylideneamino)-2-thioxoimidazolidin-4-one was synthesized according to a previously published protocol (Stanić et al., 2020).

^{1}H NMR spectra were attained on a Varian Gemini-2000 spectrometer at 200 MHz. All chemical shifts were referenced to the solvent dimethylsulfoxide-d_6 (DMSO-d_6) and downfield shifts were recorded as positive numbers.

^{1}H kinetic experiments between 3-(benzylideneamino)-2-thioxoimidazolidin-4-one and the palladium complexes were performed on fresh samples of the reactants in DMSO-d_6 at ambient temperature. The 300 μL 10 mM solutions of the thiohydantoin and complex were prepared right before the start of the experiment. After mixing the reactants, consecutive spectra were recorded with no delay, then every 5 minutes, and later on every 10 minutes, 30 minutes, 1 hour and to hours over the course of an overnight experiment.

Results and discussion

Reactions of 3-(benzylideneamino)-2-thioxoimidazolidin-4-one with $PdCl_2$, $Pd(DMSO)_2Cl_2$ and K_2PdCl_4 were monitored using a time dependent kinetic ^{1}H NMR experiment. The reactions were carried out in DMSO-d_6, because it is a suitable solvent, dissolving both the thiohydantoin and the metal complex. The

reactions were carried out in a 1:1 molar ratio, following second order kinetics. The second order reaction constants were calculated according to the equation:

$$k_2 t = \frac{x}{c_{a0}\left(c_{a0} - x\right)} \tag{1}$$

Concentrations used for calculating the rate constants were determined by integrating suitable signals in the spectra. ^{1}H NMR spectra of the reaction of 3-(benzylideneamino)-2-thioxoimidazolidin-4-one and $PdCl_2$ are shown in Figure 1.

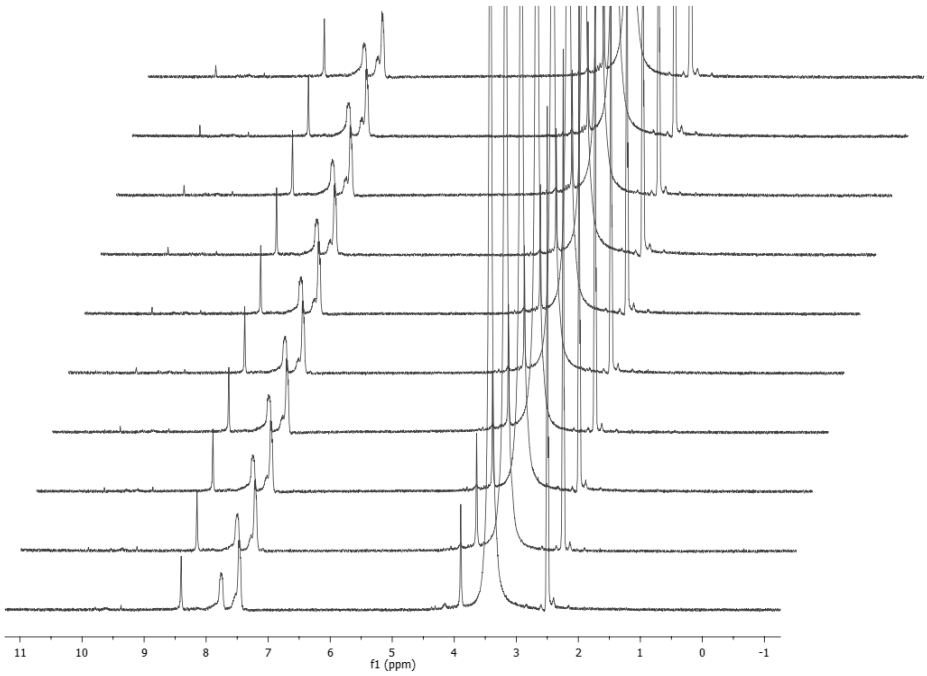

Figure 1. ^{1}H NMR spectra of the reaction of 3-(benzylideneamino)-2-thioxoimidazolidin-4-one and $PdCl_2$.

In the spectra, we can observe the absence of NH proton broad singlets. It is known that 2-thiohydantoins exist in two tautomeric forms, and it is proposed that the tautomeric form in which the thiocarbonyl sulphur exists in its thiol form is responsible for coordination (Ismail et al., 2022). The proposed mode of coordination in presented in Scheme 1.

Scheme 1. Coordination of 3-(benzylideneamino)-2-thioxoimidazolidin-4-one and $PdCl_2$.

Along with the decreasing intensity of the signals of the reacting 2-thiohydantoin (which include the ring CH_2 singlet at 3.90 ppm, aromatic protons at 7-42-8.00 ppm and the singlet of the double bond CH proton at 8.40), we can also observe a singlet that increases with intensity at 10.15 ppm that belongs to the double bond CH proton of the coordinated 2-thiohydantoin in the newly formed complex. The newly formed singlet of the double bond CH proton of the coordinated 2-thiohydantoin indicates coordination to the adjacent double bond nitrogen. The result is a five-membered chelate complex, where Pd is coordinated to the thiolic sulphur and the bouble bond nitrogen. Coordination to the thiolic sulphur is too fast for the NMR time scale to quantify, but we can observe it through the lack of an NH proton signal. Coordination to the exocyclic double bond nitrogen is the slower, rate determining step of the reaction. The corresponding rate constants were determined by integrating the singlets of double bond CH protons of the free and coordinated ligand at 8.40 and 10.15 ppm respectively. The calculated rate constant for the reaction with $PdCl_2$ is 6.83 x 10^{-5} M^{-1} s^{-1}, while for the reaction with $Pd(DMSO)_2Cl_2$, it is 3.57 x 10^{-4} M^{-1} s^{-1}. There was no reaction with K_2PdCl_4. It seems that in DMSO, chloride ions are not exchangeable ligands for this reaction. Additionally, we can observe an NH signal at 12 ppm throughout the experiment with K_2PdCl_4, since 3-(benzylideneamino)-2-thioxoimidazolidin-4-one stayed in its non-reactive tautomeric form. The reaction with $Pd(DMSO)_2Cl_2$ is faster than the reaction with $PdCl_2$ for a factor of 5. This is probably due to the labile DMSO ligands already in its structure, as $PdCl_2$ has to react with DMSO from the solvent first, forming $Pd(DMSO\text{-}d_6)_2Cl_2$, and then react with the 2-thiohydantoin derivative.

Conclusion

Reactions of an arylidene 2-thiohydantoin derivative, 3-(benzylideneamino)-2-thioxoimidazolidin-4-one with $PdCl_2$, $Pd(DMSO)_2Cl_2$ and K_2PdCl_4 were monitored in a kinetic ^1H NMR experiment. Reaction rate constants were

determined for the reactions. The reaction with $Pd(DMSO)_2Cl_2$ was faster for a factor of 5 than with $PdCl_2$, probably because of the labile DMSO ligands, while there was no reaction with K_2PdCl_4. The resulting complexes were proposed to be five-membered chelates, bonded through the sulphur atom and the exocyclic double bond nitrogen. Bonding to the sulphur is a fast process, seen only indirectly through the absence of the NH proton of the 2-thiohydantoin, because it is too fast for the NMR tiem scale. The slower, rate determining step of the reaction is coordination to the exocyclic double bond nitrogen. The results of this study could prove beneficial for a better understanding of 2-thiohydantoin coordination chemistry.

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CHEMICAL ANALYSIS OF DIFERENT BRANDS OF ORANGE JUICE IN SERBIAN MARKET

Jelena Mašković[1,], Gorica Paunović[1], Pavle Mašković[1], Ivana Kaplarević[1]*

Abstract: The chemical analysis of five different brands of orange juice in the Serbian market were evaluated to determine their overall quality. Assays were evaluated for eight quantitative characters: pH value, moisture content, total solids, acidity, content of Ca^{2+}, Mg^{2+}, Cl^- and Vitamin C at the laboratory of the Faculty of Agronomy, Čačak. The analyses were performed under the Ordinance on fruit juices and certain related products intended for human consumption ("Official Gazette of RS", No. 103/2018, 94/2019, 2/2020 - correction, 84/2020). Generally, the juice samples were within the regulatory specifications, and are fit for consumption.

Keywords: Orange juice, chemical analysis, vitamin C

Introduction

Fruits have been a part of human diet over the years. They are also considered as food supplements and are recommended internationally as essential to healthy nutrition, because they contain high quantity and quality of water, sugars, vitamins and minerals (Wardlaw, 2004; Potter and Hotchkiss, 2006). One of the ways of preserving fruits from deterioration and subsequent loss is to process them into fruit juices (Wenkam, 1990; Vanamala et al., 2006).

The sweet orange (Citrus sinensis) is native to China with the orange tree being the most cultivated fruit tree in the world. Oranges have no particular cultivation requirements, although they prefer temperate climates such as those prevailing in the Mediterranean basin.

It is well established that citrus and citrus products are a fashionable supply of vitamins, minerals and dietary fibre (non-starch polysaccharides) that are essential for traditional growth and development and over all nutritionary well-being. However, it's currently commencing to be appreciated that these and alternative biologically active, non-nutrient compounds found in citrus and alternative plants (Phytochemical) may facilitate to cut back the danger of the

[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia
(Corresponding author: jelenav@kg.ac.rs)

many chronic diseases. Whereas, applicable dietary pointers and suggestions that encourage the consumption of citrus fruit and their products will cause widespread nutritionary edges across the population. Citrus fruits are accustomed medical sciences like it improve our immune system and digestion; brighten our skin; jumpstart our metabolism; fight infection.

The objective of the present study was to evaluate the contents of the five popular brands of orange juice in the Serbian market.

Materials and methods

Five popular brands of orange juice were purchased off from different market in Serbian: Nectar family, Next joy, Life premium, Bravo and Tube. At the laboratory of the Faculty of Agronomy, volumetric methods of analysis: acid-base titration, precipitation, complexometric titration, oxidoreduction titration; and gravimetry were used for the quantitative analysis of orange juices. All samples were analyzed in triplicate. The analyses were performed under the Ordinance on fruit juices and certain related products intended for human consumption ("Official Gazette of RS", No. 103/2018, 94/2019, 2/2020 - correction, 84/2020).

Results and discussion

In the present study five brands of orange juice were evaluated for eight quantitative characters: pH value, moisture content, total solids, acidity, content of Ca^{2+}, Mg^{2+}, Cl^{-} and Vitamin C.

The results obtained from the chemical analysis of the different brands of orange juice samples are presented in Table 1.

The moisture content in the different brands of orange juice analyzed ranged from 87.05 to 92.05%. Bravo orange juice has the highest moisture content, and Nectar family orange juice has the lowest moisture content. The moisture content has an inverse relationship with the total fruit juice content.

Also, the total solids content of the fruit juices were: 12.95% for Nectar family , 10.75% for Next joy, 12.24% for Life, 7.95% for Bravo and 10.86% for Tube. The total solids and juice content are used in characterizing the quality of juice and other beverage products (Egbekun and Akubor, 2007; Adubofuor et al., 2010).

The pH value of the orange juice samples range from 2.88 to 3.73. Sample Life orange juice has the highest pH value of 3.73.

Table 1. Chemical analysis of different brands of orange juice samples

Parameter	Nectar family	Next joy	Life	Bravo	Tube
pH	3.24	2.88	3.73	3.07	2.98
Moisture (%)	87.05	89.25	87.76	92.05	89.14
Total solids (%)	12.95	10.75	12.24	7.95	10.86
Acidity (g dm^{-3})	5.79	3.54	5.36	5.47	4.29
Ca^{2+} (mg dm^{-3})	32.06	12.83	64.13	57.71	32.06
Mg^{2+} (mg dm^{-3})	77.79	38.89	136.14	42.79	27.23
Cl^- (g dm^{-3})	0.32	0.17	0.35	0.28	0.16
Vitamin C (mg dm^{-3})	506.88	270.34	473.09	337.92	304.13

Food acids dictate the dominant microflora in foods and to a large extent will determine the shelf stability of the juice (Ezeama, 2007). The more acidic the juice, the less susceptible to bacterial action but the more susceptible to the action of yeasts and moulds (Jay, 2000). Moreover Anvoh et al. (2009) reported that fruit acids influence colour, flavour and gustative characteristics of the juice products. The obtained results show that the juice produced by the Necrat family has the highest content of malic acid, and the juice produced by Next joy has the lowest content of malic acid.

Citrus fruits are rich in organic acids, which are used as a main indicator of maturation and one of the primary analytical evaluations of flavor quality when combined with sugar content. The organic acid content of fruits is especially interesting since it has a considerable influence on the sensory aspects of fruit juices, (Kelebek and Selli, 2011). Vitamin C (ascorbic acid) is an important ingredient in the diet, but it is quickly depleted or destroyed by heat and oxygen during food processing, packing, and storage. Orange juice quality is also determined by ascorbic acids, which are powerful antioxidants (Kelebek and Selli, 2011). Since of its ease of degradation, ascorbic acid is used as a reference in several industrial processes because its presence assures that the end product has a satisfactory nutritional quality (Klimczak et al., 2007). Ascorbic acid content of fruit juices is the most prominent quality index of fruit juices due to its health significance as a vitamin and cellular antioxidant (Landon, 2007). The fruit juices will contribute on the average, 40% to the recommended dietary intake of vitamin C. Sample juice produced by the Necrat family had the highest content of vitamin C (506.88 mg dm^{-3}).

The results show that the juice produced by Life has the highest content of chloride ions (0.35 g dm^{-3}), and the lowest content of chloride ions has the juice of Tube (0.16 g dm^{-3}).

The highest concentration of Mg^{2+} is in the sample produced by Life, and the lowest concentration is in the sample produced by Tube. The presence of Ca^{2+} is the highest in the manufacturer Life, and the lowest in the manufacturer Next joy.

All the obtained results are in accordance with the Ordinance on fruit juices and certain related products intended for human consumption ("Official Gazette of RS", No. 103/2018, 94/2019, 2/2020 - correction, 84/2020).

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Conclusion

In the present study five brands of orange juice were evaluated for. The results of chemical analyzes of basic quality indicators were used. The analyzes were done at the Laboratory of the Faculty of Agronomy in Čačak. All the obtained results are in accordance with the Ordinance on fruit juices and certain related products intended for human consumption ("Official Gazette of RS", No. 103/2018, 94/2019, 2/2020 - correction, 84/2020). The difference in the quality attributes of the different brands of orange juice may be attributed to the different processing procedures employed and storage conditions. Consumption of these beverages is desirable as they would serve as good sources of vitamins and body electrolytes.

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CHEMICAL COMPOSITION OF LEMON GRASS EXTRACTS

Jelena Mladenović[1], Đorđe Jovanović[1], Nenad Pavlović[1] , Milena Đurić[1] , Ljiljana Bošković-Rakočević[1],Jasmina Zdravković[2]

Abstract: The plant lemon grass (*Aloisia citrodora*) was used as material in this work. The percentage of dry matter, organic acids and cellulose, was determined from the fresh plant material. Extracts are obtained from chopped dry lemongrass leaves. Extraction was done by maceration, Soxhlet and ultrasound. The content of extracted matter in the obtained extracts was determined, as was the content of vitamin C.

Keywords: lemon grass, extraction, organic acids, vitamin C.

Introduction

The most important group of herbal preparations is represented by extracts, which are obtained by applying different extraction methods, starting from simpler technologies to advanced techniques. Extraction is the separation and concentration of certain constituents from plant and animal tissues using selective solvents using standard procedures, (Lampe,1999). Depending on the consistency, extracts are divided into liquid, semi-solid and solid. Plant extracts are obtained by bringing chopped, mostly dry material into contact with an extraction solvent in a suitable device, an extracto, (Damjanović, 2007).

Lemongrass is a perennial evergreen plant from the verbena family (*Verbenaceae*). It has a bushy growth, well branched, up to 3 m tall. The leaves are opposite or clustered in groups of three, up to 8 cm long, green and shiny, when crushed between the fingers they release a pronounced lemon scent. The flowers are small, white or purple, fragrant gathered in oblong, branched spikes about 10 cm long, (Lakušić, 1990).

The leaves are used as tea or as a spice for flavoring drinks, marinades, creams, ice cream, cakes. They can be used fresh or dried, and by drying they

[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia (jelenamala@kg.ac.rs)
[2]Institute for forage crops, 37000 Kruševac, Serbia (jasna.zdravkovic@gmail.com)

retain their aroma for many years. It is used for stress, depression and in the production of perfumes and some hygiene products.

Materials and methods

A plant, lemon grass (*Aloysia citrodora*) was used as a material in this final work. Plant material for analysis was collected in October 2017 in Crete. The percentage of dry matter was determined from the fresh plant material, i.e. content of water, organic acids and cellulose. The plant was dried in the shade and stored in a dry place. Extracts are obtained from chopped dry leaves of lemon grass.

In laboratories, the determination of water content by drying is most often applied. Three measurements of the crushed sample were performed. The samples were dried to a constant mass in a dryer under atmospheric pressure at a temperature of 105 ^{0}C. Before the samples were placed for drying, the Vegeglas was dried with a lid in an oven to a constant mass (1 hour) at the prescribed temperature (105 ^{0}C), (Piletić i Miletić, 1989).

The examined sample was placed in a dry Vegeglas and measured. Then it was put to dry in a dryer. During drying, the Vegeglas with the sample must be open, i.e. the lid is next to it during drying. After drying, vegeglas is cooled in a desiccator, and then measured.

The process of drying, cooling and measuring was repeated until a constant mass. Since three measurements were made, the mean value was recalculated. The moisture content is obtained from the difference in mass before and after drying the tested sample. The mean value is recalculated for three measurements.

Cellulose content was determined by the Scharrer-Kürschner method, which involves destroying the sample with a mixture consisting of nitric, acetic and trichloroacetic acids. Nitric acid oxidizes and nitrates all substances, except cellulose, and the decomposed products are dissolved in acetic acid (Šiler-Marinković, 2009).

In an Erlenmeyer with a ground neck, 1.00 g of the cause is transferred and then poured with 25 cm^3 of the reagent for cellulose, connected to the return condenser and heated for 30 minutes on an electric heater, over an asbestos mesh. It is necessary to periodically stir the contents of the Erlenmeyer flask in order to remove particles from the vessel walls. After cooking, the content is filtered through previously dried and weighed filter paper. The precipitate is washed with hot water and ethanol, then dried at 105 °C to a constant mass, cooled in a

desiccator and measured. From the difference in mass of filter paper with cellulose and filter paper, the amount of cellulose (including mineral substances) is obtained.

The determination of organic acids was carried out by the volumetric neutralization method, where the solution is titrated using a base solution (NaOH) of known concentration, in the presence of the phenolphthalein indicator, (Milić i sar., 2012).

The test sample, 100g, is crushed in a porcelain mortar. With the addition of water, the sample is quantitatively transferred into a measuring vessel of 250 mL, and all this is diluted with distilled water to 150 mL. After that, the extraction is carried out, which is accelerated by heating in a water bath at a temperature of 70-80°C with occasional stirring for 30 minutes. The obtained extract is then cooled, the measuring vessel is filled with distilled water up to the line and then filtered through pleated filter paper. After filtration, 50 mL of the filtrate with dissolved acids is transferred with a pipette into a 250 mL Erlenmeyer flask, 2-3 drops of 1% phenolphthalein are added and titrated with a 0.1 mol/L NaOH solution.

Free acidity is expressed in g/100g of fresh sample through citric acid, which is dominant in the sample.

Extraction by maceration

Chopped and homogenized plant material (5 g) was poured with solvent (250 mL 96% ethanol) and left in well-closed Erlenmeyer flasks, protected from light. After 5 days, the plant material was separated from the macerate by straining through cheesecloth, and then through filter paper, a black strip. The solvent was removed by evaporation on a water bath, and the obtained extract was dried to a constant mass, (Lajšić i Grujić-Injac, 1998).

Soxlet extraction – y

A measured mass of chopped and homogenized plant material (5 g) was placed in a sleeve. The sample sleeve was then inserted into the middle part of the extractor which was connected to the cooler and balloon. Erlenmeyer was previously dried for 1 hour at 105 ^{0}C and weighed on an analytical balance. Using a small funnel from the top of the condenser, enough solvent was poured into the apparatus to fill the extractor and pour into the Erlenmeyer flask. Then a little more solvent (96% ethanol) was added, making sure that the total amount of solvent did not occupy more than ¾ of the Erlenmeyer volume, (Milić i sar., 2012).

The apparatus was placed on a rack and the solvent was gradually heated in an Erlenmeyer so that the condensed drops of solvent falling on the hilzne could be counted rather than leaking in a continuous stream. The extraction was performed at the boiling temperature of the solvent for 6 hours. After the extraction was completed, the extracted substance was dissolved in the solvent in an Erlenmeyer

flask. The solvent was removed by evaporation and then the extract was dried in an oven to a constant mass.

Ultrasonic extraction

Ultrasonic extraction was performed in an ultrasonic water bath (EUP540A, Euinstruments, France) [12]. The sample (5g) was placed in a flask and poured with 250 mL of 96% ethanol. The mixture was extracted for 30 minutes at a frequency of 40 kHz and an ultrasound power of 90% (216 W), (Milić i sar., 2012).

Determination of vitamin C content

Quantitative determination of total vitamin C is based on the reversible ability of the oxidation-reduction system ascorbic-dehydroascorbic acid, (Aćamović-Đoković i Cvijović, 2009).

The Tillmans method was used for the quantitative determination of vitamin C, which is based on oxidometric titration during which L-ascorbic acid is oxidized to dehydroascorbic acid, with simultaneous reduction of the applied reagent. Titration with 2,6-dichlorophenolindophenol. Tillmans reagent (TR) is performed in an acidic medium at pH 4–6. The oxidized form of the Tillmans reagent solution (which also acts as an indicator) has a dark blue color (at pH 5.2), while in the presence of ascorbic acid, TR changes to its reduced, leuco form. At pH 4.2, TR has a red color (acidic environment), and when all the amount of L-ascorbic acid is oxidized, the next drop of TR colors the tested solution pink because the reaction environment is still acidic, (Džamić, 1984).

For the extraction of ascorbic acid from the plant extract, 10% acetic acid or 5% metaphosphoric acid or their mixture is used. These acids favor protein precipitation and at the same time slow down the reaction of other reducing substances with Tillmans reagent; also, they keep the environment acidic.

Results and discussion

The content of dry matter obtained on the basis of three measurements was calculated as their mean value and is 27.354%, and the moisture content obtained by subtracting the content of dry matter per 100 g of the sample, for each measurement also expressed as their mean value, is 72.645%.

The content of organic acids, as well as the content of cellulose, was determined in fresh plant material calculated on dry matter. Analyzes were performed in three trials and converted to the mean value. The mean value of organic acid content is 3.19%, while the mean value of cellulose content is 35.51%.

Table 1. Content of organic acids and cellulose

Organic acids, (%)	Cellulose,(%)
3.20	35.35
3.15	35.43
3.22	35.75

After the extractions have been completed, the extraction yield is calculated by evaporating the obtained plant extracts to dryness and measuring the obtained dry residues. 0.4215g of dry macerate matter, 0.2545g of Soxhlet extract and 0.695g of ultrasonic extract were obtained from 5g of plant material of lemongrass leaf. The obtained results in percentages are shown in Table 2.

Table 2. Percentage yield of density extraction of obtained extracts

	maceration	Soxhlet extraction	Ultrasonic extraction
extraction yield, %	8.43	5.09	13.09
density of extracts, g/cm^3	0.75	0.68	0.82
Vitamin C, mg / 100 g	9.5	4.5	17.5

Based on the obtained results, it can be concluded that the lowest yield was obtained by Soxhlet extraction, followed by maceration, while the highest yield was obtained by ultrasonic extraction, which proved to be the extraction method with optimal conditions for the lemongrass plant. Given that this plant contains a lot of vitamins and bioactive compounds, we assume that during Soxhlet extraction, the mentioned thermolabile compounds were decomposed, and during maceration, the length of extraction affected their decomposition.

Based on the density measurement, we see that the highest density in the ultrasonic extract is 0.82 g/cm^3, and the lowest in the Soxhlet extract is 0.68 g/cm^3, which correlates with the extraction yield.

When determining the content of vitamin C, we concluded that the highest content of this vitamin was determined, also in the ultrasonic 17.5 mg/100g, then in the macerate 9.5 mg/100g, and the lowest in the Soxhlet extract 4.5 mg/100g. With ultrasonic extraction, we managed to isolate the largest amount of this vitamin because the temperature in the bath was 40 C^0, which is a lower temperature than the decomposition temperature of this vitamin (50-60 C^0), in

Soxhlet extraction the temperature of the solvent was at the boiling point, i.e. 60 C^0, and with maceration even if the solvent was at room temperature, the process lasted 5 days, which probably led to the breakdown of vitamins. We assume that the ultrasonic extraction method proved to be the most optimal with the highest yield because it lasted the shortest (30 min), at the lowest temperature. In this way, the vitamin was quickly extracted and preserved from degradation.

The plant, lemongrass, was not examined chemically, but by comparing the values of the content of organic compounds in the plant as well as the content of water, the obtained values are within the limits of the expected results (MacVicar J.,2006).

Conclusion

When determining the content of vitamin C, we concluded that the highest content of this vitamin was determined, also in the ultrasonic 17.5 mg/100g, then in the macerate 9.5 mg/100g, and the lowest in the Soxhlet extract 4.5 mg/100g. With ultrasonic extraction, we managed to isolate the largest amount of this vitamin because the temperature in the bath was 40 C^0, which is a lower temperature than the decomposition temperature of this vitamin (50-60 C^0), in Soxhlet extraction the temperature of the solvent was at the boiling point, i.e. 60 C^0, and with maceration even if the solvent was at room temperature, the process lasted 5 days, which probably led to the breakdown of vitamins. We assume that the ultrasonic extraction method proved to be the most optimal with the highest yield because it lasted the shortest (30 min), at the lowest temperature. In this way, the vitamin was quickly extracted and preserved from degradation.Please check each item to be certain that the article is in compliance with the requirements.

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COMPARISON OF COW'S MILK WITH PLANT-BASED MILK ALTERNATIVES: SELECTED CHEMICAL AND PHYSICAL ANALYSIS

Vesna Milovanović[1], Miloš Petrović[1], Vladimir Kurćubić[1], Marko Petković[1], Nemanja Miletić[1], Igor Đurović[1]

Abstract: The aim of this paper is to make a chemical and physical comparison of cow's milk with plant-based milk alternatives. Selected chemical and physical parameters of animal milk (raw and pasteurized cow's milk) and plant based milk (almond and soy milk) were done. The titratable acidity, pH, conductivity, viscosity and density were measured by using standardized techniques. Additional parameters such as proteins, fat, (solids-not-fat) SNF, and lactose were determined by milk analyser and compared. The results showed that plant-based milks contain low values of proteins, fat, SNF and significantly lower acidity in comparison with cow's milk.

Keywords: raw cow's milk, pasteurized cow's milk, soy milk, almond milk

Introduction

Milk is one of the most frequently consumed foods due to its key role in nourishment and hydration. Cow's milk has been the subject of detailed chemical or nutrient analyses for many years. These include numerous studies on milk proteins, fats, carbohydrates, vitamins, and minerals (Foroutan et al., 2019). At the macronutrient level, cow's milk is composed of water (85-87%), fats (3.8-5.5%), carbohydrates (5%), and proteins (2.9-3.5%). At a micronutrient level, cow's milk contains various bioactive compounds including vitamins, minerals, organic acids, biogenic amines, oligosaccharides, nucleotides, and immunoglobulins (Fox et al., 2015).

Functional beverages have been related to several of health benefits, thus, alternatives to bovine milk beverages are in expansion, primarily made from plant sources such as soy, almond, coconut, rice, and nuts (Vallath et al, 2022). Due to health consequences or because of lifestyle choices (flexitarian, vegetarian, and vegan lifestyles), consumer demand for cow's milk alternatives

[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia (igor.djurovic@kg.ac.rs)

has increased, leading to a bigger market share of plant-based milks (PBM) (Antunes et al., 2023). The organisation of the European single market for agricultural products (Regulation (EU) 1308/2013, also known as the CMO) defines „milk products" as products that are exclusively derived from milk, where "milk" presents "mammary secretion obtained from one or more milkings". The CMO specifies that dairy terms such as milk, butter, and yoghurt are completely reserved for milk and milk products. Dairy alternative products therefore cannot be referred to with dairy-associated names. For example, soy-based drinks are not allowed to be called 'soy milk' in the EU, as the product is not mammary secretion and this term is described on the list of exceptions under Commission Decision 2010/791/EU (Leialohilani, 2020).

PBM are completely free from animal-based ingredients and lack certain components associated with mammalian milk such as cholesterol, saturated fatty acids, antigens and lactose while on the same time being the good source of non allergic proteins, minerals, essential fatty acids etc., making it suitable as dairy free alternative (Das et al., 2012). PBM present fluids derived from maceration of plant material (legumes, nuts, or cereals) soaked in water and homogenisated to a size range of 5-20 μm (Kundu et al., 2018).

In general classification there are five categories of the PBM alternatives:
(1) Cereal based (Rice milk, Oat milk, Corn milk, Spelt milk); (2) Pseudo-cereal based (Quinoa milk, Amaranth milk, Teff milk); (3) Nut based (Almond milk, Coconut milk, Hazelnut milk, Pistachio milk, Walnut milk); (4) Legume based (Soy milk, Peanut milk, Lupin milk, Cowpea milk); (5) Seed based (Sesame milk, Sunflower milk, Flax milk, Hemp milk) (Sethi et al., 2016; Pointke et al., 2022).
Soy milk and almond milk are rich creamy white liquids which display similarities to cow's milk in appearance and consistency (Kundu et al., 2018).

Soy milk presents healthy food drink containing high amounts of protein, iron, unsaturated fatty acids, and niacin, but low amounts of fat, carbohydrates, and calcium comparing with cow's milk. Additionally, soy milk has high concentration of fosfatidylcholine and vitamins as well as isoflavones which are potent antioxidants (Mazumder et al., 2016).

Compared to other PBM, almond milk is naturally a rich source of vitamins, especially vitamin E which presents an esential vitamin and need to be supplied through diet or supplements (Sethi et al., 2016). Almond milk is rich source of nutrients dense lower calories than soy and cow's milk. Its nutritional benefits promote cardiovascular health and reducing of mineral deficiencies especially iron and zinc (Yetunde et al., 2015).

Materials and methods

Milk sample collection: Commercially available PBM were compared to cow's milk (raw and pasteurized form). Almond milk and soy milk were purchased from a local market. Raw cow's milk was purchased from the farmer. Cow's milk was analysed in raw and pasteurized form. Pasteurization was done by heating raw milk at 60 °C in 30 min.

Chemical analysis: Proteins, fat, SNF, and lactose content were determined by milk analyser Julie C8 automatic™ Scope Electric. The acidity of milk was determined by Soxhlet-Henkel method, titrating the sampe with standardized 0.25 N NaOH, using 2% phenolphthalein as the indicator, giving an end-point of a faint pink color. Results are presented in Soxlet Henkel degrees (°SH). The acidity was also expressed as % of lactic acid - each degree Soxhlet-Henkel is equivalent to 0.0225% lactic acid in the milk (Teichert et al., 2020).

Physical analysis: The pH and conductivity of milk samples were determined by CyberScan CON 510-portable conductivity meter. Viscosity was determined by Ostwald's viscometer at 20 °C. The time in seconds was converted into dynamic viscosity by using the diameter of the capillary and the formula:

$$\eta_x = \eta_{H_2O} \cdot \frac{\rho_x \cdot t_x}{\rho_{H_2O} \cdot t_{H_2O}}$$

Where ρ_x and ρ_{H_2O} are densities of examined samples and water and t_{H_2O} is the coefficient of viscosity of water at 20 °C (Atkins, 1990). Density was determined by lactodensimeter at 20 °C. All measurements were performed in triplicate. Results are represented mean values of three independent measurements ± standard deviation (SD).

Results and discussion

In order to compare PBM with cow's milk, selected chemical and physical analysis were performed, Table 1. Almond and soy milk were selected as PBM alternatives as the most commonly used by consumers, and cow's milk was analysed in raw and pasteurized form. Pasteurization of milk maintains the content of protein and lactose similar to raw milk and slightly changes the composition of milk, decreasing total fat and total solids (Pestana et al., 2015). Our results showed that pasteurization of milk did not result in significant changes of the examined parameters except in the case of fat content which decreased after pasteurization (5.58% in raw cow's milk and 3.57% in

pasteurized milk), Table 1. Comparing PBM, soy milk contains a higher content of proteins, fat, and SNF which is in agreement with literature data (Collard et al., 2020). As it is expected and in accordance to literature (Sethi et al., 2016), cow's milk is ahead of PBM when it comes to the amount of protein, fat, SNF and, lactose, Table 1.

Table 1. Selected chemical and physical characteristic of Cow's Milk, Almond and Soy milk

Parameters	Raw Cow's milk	Pasteurized Cow's milk	Almond milk	Soy milk
Proteins (%)	2.81±0.06	3.19±0.08	0.08±0.01	1.83±0.01
Fat (%)	5.58±0.09	3.57±0.06	0.67±0.03	1.42±0.01
SNF (%)	7.66±0.16	8.70±0.22	0.21±0.02	4.98±0.02
Lactose (%)	4.21±0.08	4.78±0.13	0.11±0.01	2.74±0.01
Acidity (°SH)	7.88±0.18	8.63±0.18	0.50±0.01	2.38±0.18
Lactic acid (%)	0.18±0.01	0.19±0.01	0.01±0.01	0.05±0.01
pH	6.63±0.04	6.73±0.04	8.23±0.03	8.27±0.04
Conductivity (mS)	3.27±0.04	3.49±0.05	1.22±0.02	1.95±0.07
Density (g/cm^3) at 20 °C	1.029±0.012	1.026±0.021	1.009±0.012	1.019±0.013
Viscosity (Pa s) at 20°C	1.704±0.022	1.725±0.024	4.260±0.021	2.000±0.032

Results represent mean values ± standard deviation (SD) of three independent measurements.

By comparing the acidity of milk (°SH and lactic acid %), higher values were observed in cow's milk than in PBM and that is expected due to the presence of lactic acid. This is also confirmed by the value for pH, which is lower in cow's milk samples. The results showed higher conductivity of cow's milk than PBM. The biggest contribution to conductivity comes from milk salts which some are dissolved as ions, while others, depending on temperature and pH, are in equilibrium with different species associated with casein micelles or other proteins and their contribution to conductivity is difficult to determine (Henningsson et al., 2014). Lower values for density in PBM compared to cow's milk were expected considering the lower percentage of fat, protein, and SNF, and therefore a higher percentage of water.

From the value for viscosity, it can be concluded that PBM have a higher viscosity due to pronounced force of internal friction between the liquid layers which is in agreement with literature data (Mäkinen, 2015). The highest viscosity is found in almond milk, which is attributed to the lowest content of fat, protein and SNF, which leads to increase in the forces of internal friction between polar molecules of water.

Conclusion

PBM present excellent alternatives for cow's milk especially in case of cow's milk allergy, lactose intolerance, calorie concern, hypercholesterolemia, and diabetes. In addition, PBM is an excellent choice for food preparation during fasting.

The results of this study show that PBM alternatives contain low values of proteins, fat, SNF and significantly lower acidity in comparison with cow's milk.

This study is definitely not the final word on the chemical and physical composition of cow's milk and PBM alternatives. In the future, testing of a larger number of samples, additional physical and chemical analyses, as well as sensory evaluation are planned.

Acknowledgement

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THE INFLUENCE OF THE ENOLOGICAL TANNINS APPLICATION ON THE PHENOLIC COMPOSITION OF WINE

Aleksandar Petrovic[1], Nikolina Lisov[1], Ivana Plavsic-Janjatovic[1], Ivana Sredovic-Ignjatovic[1], Danka Mitrovic[1]

Abstract: The chemical composition of wine depends on the degree of maturity and quality of the grapes, as well as the agroecological conditions under which the grapes are grown. Tannins are of particular importance for wines, they are the source of the characteristic astringency of red wines. The main aim of this study was to investigate the influence of addition of enological tannins (0.05 g/L; 0.15 g/L; 0.3 g/L) on the phenolic composition of wine. The effect of the addition of differenttannins on the certain phenolic compounds (gallic acid, caffeic acid, ellagic acid and quercetin) was measured by HPLC analysis.Only forcontent of gallic and caffeic acid, there was a statistically significant difference between the control sample and all other samples with added tannins.

Keywords: enological tannins, phenolic compounds, wine, HPLC analyse

Introduction

The grape harvest is done when technological and phenolic maturity is reached. These two maturities coincide in good years, but in bad years the difference between full technological and phenolic maturity can be as much as 15 days.

Different oenological supplies, such as chips, staves and commercial enological tannins, are widely used to add phenolic compounds to wine (Vazallo-Valleumbrocio et al., 2017). With the development of tannin products, newoenological tannins are developed with many specific functions, such as modifying antioxidanteffect, colour stabilization and aroma modifications (Chen et al., 2016). According to their structure, tannins can be broadly divided into two classes of macromolecules, termedhydrolysable tannins and condensed tannins (Fig. 1) (Herderich and Smith, 2005). The action mechanism is different depending onthe nature of the tannin. Condensed tannins(procyanidins) can combine with anthocyanins(directly or by means of acetaldehyde mediatedreactions) and stabilize wine colour. Hydrolysabletannins cannot participate in condensation reactions with anthocyanins, but they can participatein copigmentation reactions, as well as protectwine anthocyanins from oxidation since they mayregulate

[1]University of Belgrade, Faculty of Agriculture, Nemanjina 6, Belgrade, Serbia (aleksandar.petrovic@agrif.bg.ac.rs)

oxidation-reduction phenomena (Bautista-Ortin 2005).Hydrolysable tannins are generally extracted from the oak barrelsused in wine ageing. Their structure consists of a glucose molecule acylated with galloyl groups. The quantity of these oak derived tannins depends on the amount of time the wine is agedin the barrels, whether the barrels were new or had been usedpreviously and the origin of the oak (Smith et al., 2015).Hydrolyzable tannins are gallotannins and ellagitannins and are suitable for repairing the wine structure. These are polyesters of gallic acid or its derivatives and the central sugar molecule, usually glucose. Gallotannins are esters of gallic acid and glucose. Gallic acid is a natural constituent of grape seeds and skins and is always present in wine. Ellagitanins are esters of ellagic or hebulic acid and glucose. Ellagic acid is not a natural ingredient of wine, but is found in wine if it has been aged in oak barrels. Ellagitannins are non-volatile components of oak, in: castalagin, vescalagin, grandinin, roburin A, B, C, D, and E. Ellagitannins have a great influence on wine stabilization because they are involved in oxidation, copigmentation and polymerization reactions with anthocyanins and oxygen (Ribéreau-Gayon et al., 2006).

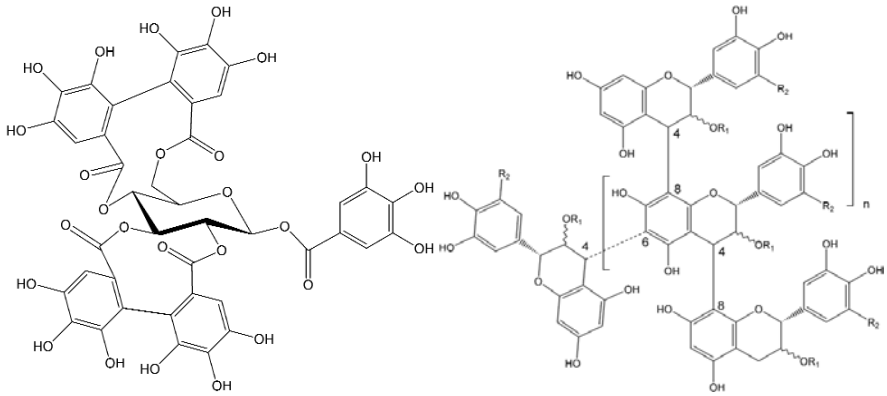


Figure 1. Examples of hydrolysable tannins (left) and condensed tannins (polymeric flavan-3-ols, right) (Herderich and Smith, 2005)

Materials and methods

Primary grape processing and vinification

The grapes originate from the vineyard in Vojnica near Veles (North Macedonia). The grapes were harvested in a state of technological maturity, phytosanitary state – 100% healthy.

After grapes crashing and destemming, the samples were sulfited with 10 g of $K_2S_2O_5$ per 100 kg, pectinolytic enzymes ExV (Lallemand, Canada) in the amount of 2 g/kg and yeast strain *Saccharomyces cerevisiae* in the amount of 20 g/hl (BDX, Lallemand, Canada) were inoculated.Alcoholic fermentation and maceration was carried out at an average temperature of 25°C, with pomace punching down twice a day. The young wine was separated from the pomace after 21 days of maceration. After a year of aging in a stainless steel vessel, the samples were bottled. A wines were aging for a 6 months in the bottle, and after that increasing concentrations of three different enological tannins were added.

Addition of enological tannins

The experiment was set up by preparing a 5% solution of used enological tannins in red wine(2 condensed:Tanenol elegance and Tanenol elevage, and 1 hydrolyzable:Tanenol fruitan). During dissolving of condensed tannins, thickening occurred, while this was not the case with hydrolysable ones. This is a consequence of the rapid reaction of tannins with proteins from the wine. After stabilization, tannins solutions were added to wine (750 ml bottles) in concentration of 0.05 g/L; 0.15 g/L; 0.30 g/L (Table 1). The bottles were exposed to aging at a constant temperature of 14 -16 ºC for a year.

Tanenol elegance (Enartis, Italy)

Condensed tannin obtained from tannins extracted from the skin of white grapes. Tanelol elegance is a light reddish skin-colored powder. It has a slight scent of chamomile and grapes. It represents a mixture of condensed tannins made mainly from fine tannins from the skins of well-ripened and carefully selected white grapes. It improves the color as well as the content of polyphenols, affects fruitiness, fullness and complexity. It can also be used in the aging and maturation phase where it contributes to the overall organoleptic properties, intensity and extended finish.

Tanenol elevage (ENARTIS, Italy)

It is a fine, amorphous, light brown powder with a slight woody smell. Tanenol Elevage is an ellagitannin extracted from the oak tree. It is used in vinification as well as a corrective agent. It improves the structure, aroma and balance of taste in both white and red wines. The addition of this tannin at the end of malolactic fermentation protects the molecules responsible for the color

from oxidation, also affects: color stabilization in wine and spreads, improvement of the structure, for the formation of a complex of sulfur compounds due to prolonged contact with the sediment.

Tanenol fruitan (Enartis, Italy)

Condensed tannin, extracted from white grape seeds. Tanenol fruitan are creamy beige granules with a fine grape aroma. It represents a mixture of condensed tannins, mostly extracted from fresh, physiologically ripe white grape seeds. These proanthocyanidic tannins interact with anthocyanins (the substances responsible for the color of red wines), binding them and thus protecting them from oxidation. The use of Tanenol fruitan during the fermentation enables better development and better maintenance of the color and improved stability of the color of the wine over time. In red wines, Tanenol fruitan has a positive effect on the body and fresh fruity character of the wine. structure facilitates easy dispersion in water and wine and thus facilitates the work of the cellar because it does not create lumps.

Table 1. Presentation of tannins that were added and in which concentrations

Sample number	**The name of the added tannin**	**Amount of added tannin**
1.	Tanenol elegance	5 g/hL
2.	Tanenol fruitan	5 g/hL
3.	Tanenol elevage	5 g/hL
4.	Tanenol elegance	15 g/hL
5.	Tanenol fruitan	15 g/hL
6.	Tanenol elevage	15 g/hL
7.	Tanenol elegance	30 g/hL
8.	Tanenol fruitan	30 g/hL
9.	Tanenol elevage	30 g/hL
CS*	Control sample	--

*Control sample

Total phenolic compounds

Total phenolic content in wine samples was determined by the Folin–Ciocalteu's (FC) method using gallic acid as a standard.Total phenolic

compounds were determined by the Folin-Ciocalteu method with gallic acid as a standard, and the results were expressed in g/L of gallic acid. The solution was prepared by adding 1 ml of wine, 5 mL of FC reagent and 10 ml of saturated sodium carbonate to 75 mL of water. The reaction takes place at room temperature for one hour. After this time, the absorbance at λ = 720nm was measured for each sample and blank. Values for total phenols were obtained from the diagram(Tanner and Brunner, 1979).

HPLC analysis

The determination of tannin content in wine was performed on a Waters Acquity UPLC H-Class liquid-mass chromatograph (WAT-176015007) equipped with a Waters Quaternary Solvent Menager quaternary pump, a Waters Sample Menager-FTN (Flow Through Needle) injector, a column compartment with a ZORBAX Eclipse XDB C18 column (150 × 4.6 mm; 5 µm), Waters 2998 PDA (Photodiode Array) detector and Waters TQ mass detector (Tandem Quadrupole, WAT-176001263). MassLynx V4.1 software was used for data acquisition and processing. HPLC grade acetonitrile, HPLC grade formic acid and deionized water were used (Table 2).

Wine samples were filtered through an Econofilter 25/0.45 µm, Agilent Technologies and passed through a ZORBAX Eclipse XDB C18 column (150 × 4.6 mm; 5 µm). Liquid chromatograph conditions:

Table 2. Liquid chromatograph conditions: flow of mobile phases 0.7 mL/min; column temperature 25 °C; liquid chromatogram and UV spectra were recorded at wavelengths ranging from 190 nm to 600 nm; The injection volume is 10 µL.

Time (min)	0,2% formic acid in water	Acetonitrile %
0	95	5
20	84	16
28	60	40
32	30	70
36	0	100
45	0	100
46	95	5
55	95	5

The MS Scan of the tannin standard was recorded in ESI- and ESI+ mode.

Conditions for recording mass spectra:

The m/z 229→107 transition was monitored in ESI+ MRM mode (Multiple Reaction Monitoring Mode) under the following conditions: capillary voltage 3.5 kV, cone voltage 34 V, collision energy 24 eV, source temperature 150 °C, desolvation temperature 450 °C, desolvation gas flow 800 L/h.

The samples were recorded without prior preparation. They were filtered through an Econofilter 25/0.45 μm filter.

Results and discussion

Regarding to results for gallic acid, there was a statistically significant difference between the control sample and all other samples with added tannins (Table 3). Also, it was noticed a significant statistical difference for gallic acid content between the wine samples to which the enological tannins of elegance and fruitan were added ($p<0.05$). Addition of all applied enological tannins led to increasing of gallic acid content what was consistent with other studies (Bautista-Ortín et al., 2005; Bautista-Ortín et al., 2007). According to Li et al. (2020), the contents of gallic acid and caffeic acid increased with the addition of any testedtannin what was in agreement with our research. Contrary to these results, wine treatments with three different concentrations of tannins (100, 250 and 400 ml/L of Tanin VR GRAPE preparation) didn't show any effect on the phenolic acids, exactly gallic and caffeic acid (Ghanem et al., 2017).Regarding the caffeic acid content, there was a statistically significant difference between the control sample and samples to which tannins were added. Different concentrations of added tannins did not significantly affect content caffeic acid ($p>0.05$). For ellagic acid content, there was no statistically significant difference between the control sample and the samples with the addition of tannins, as well as when comparing different tannins and their different concentrations ($p>0.05$). In study of Li et al. (2020), addition of condensed and hydrolysable tannins did not significantly changed content of ellagic acid. In terms of quercetin content there was no statistically significant difference between the control sample and all other variants of the experiment.Comparing the addition of different oenological tannins and their different concentrations to the content of quercetin, there was no statistically significant difference ($p>0.05$). Contrary to our results, Bautista-Ortín et al. (2007) noted that in wine elaborated with the addition of different enological tannins, content of quercetin was a higher than its content in control sample.

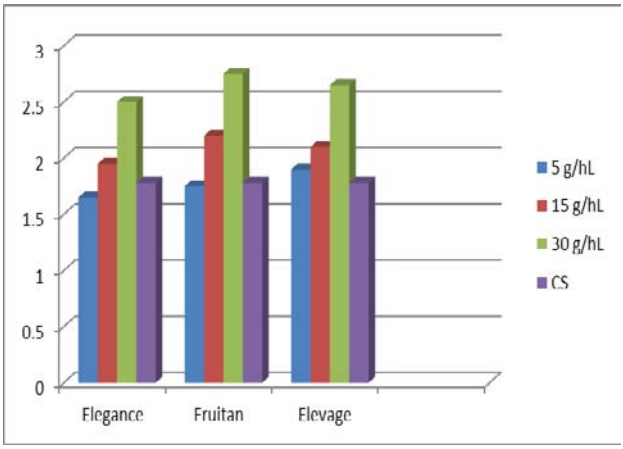
Table 3. Content of certain phenolic compounds measured by HPLC analysis

Samplenumber	Gallic acid mg/L	Quercetin mg/L	Caffeic acid mg/L	Ellagic acid mg/L
1.	726.64	9.36	45.97	6.75
2.	551.47	10.21	41.87	6.19
3.	513.84	10.34	40.02	8.32
4.	501.86	7.54	38.09	6.99
5.	406.79	9.23	37.40	6.37
6.	497.62	9.16	44.39	11.72
7.	602.28	10.58	34.51	5.01
8.	392.13	8.69	31.98	15.78
9.	355.83	8.10	30.51	1.58
*CS	345.53	9.62	0.09	8.09

*Control sample

Total phenolic content in wines

In terms of total phenolic content in wines with added tannins it was shown its increasement compared to control, which was no statisticaly significant ($p>0.05$). The highest content was noticed in wine sample to which was added Tanenol fruitan in the highest concentration (Graph 1) and the lowest one in the control sample (1.78 g/L). According to Vazallo-Valleumbrocio et al. (2017) analysis of the wines demonstrated that the use of any of the commercial enological tannins modified the chemical composition of the wines. So, wine with no addition of enological tannins presented the lowest total phenolic content (Vazallo-Valleumbrocio et al., 2017), what was consistent with our research. The addition of tannins especially at high concentration leads to a significant increase in total phenolic content compared to the control sample (Ghanem et al., 2017) what is opposite to our results.



Graph 1. The results for total phenolic content

Conclusion

Tannin addition is an enological practice widely widespread because of many economical and benefits related to wine quality. Addition of enological tannins for red wines had a positive effect in terms of content of analysed phenolic compounds. A higher concentration of added tannins lead to higher content of certain and total phenolic compounds. Furthermore, the type and amounth of tannins chosen to be added should be based on the characteristics of the wine.

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UPTAKE OF HEAVY METALS BY ALFALFA (*Medicago sativa* L.): POT EXPERIMENT

Denis Mitov[1], Stefan Petrović[1], Aleksandar Ranđelović[1], Jelena Mrmošanin[1], Aleksandra Pavlović[1], Snežana Tošić[1]

Abstract: In this work, the uptake of some heavy metals (Cd, Cr, Cu, Ni and Pb) by alfalfa (*Medicago sativa* L.) was studied in a pot experiment. Alfalfa and soil samples were prepared by wet digestion and analyzed by ICP-OES. The obtained results were discussed based on the determined concentrations, translocation factors (TFs) and enrichment factors (EFs) values, showing that this plant species is able to uptake increased amounts of Cd by roots.

Keywords: alfalfa, heavy metals, pot experiment, ICP-OES determination

Introduction

Alfalfa (*Medicago sativa* L.) is a widely grown perennial plant used for hay, silage, and pasture. The advantage of growing alfalfa is that it prevents soil erosion and improves the arable quality of the soil, as it has a deep root system and is also a perennial plant. This plant participates in the process of fixing atmospheric nitrogen, thus providing sufficient amounts of nitrogen in the soil (Li and Brummer, 2012). Alfalfa is a good source of carbohydrates, proteins, minerals, fat-soluble and water-soluble vitamins, making it suitable for feeding domestic animals, especially ruminants (Mielmann, 2013). In addition, there are a growing number of studies focusing on the medicinal effects of alfalfa, such as antioxidant, anti-inflammatory, antimicrobial, cytotoxic and similar (Al-Snafi et al., 2021).

Heavy metals are naturally present in soil, but in recent decades, the amount of heavy metals in soil has increased significantly due to various human activities. Heavy metals can have different effects on plants and other organisms. Essential metals are necessary for normal plant growth and development. Some of the non-essential metals may be toxic or potentially toxic and may have adverse effects on plants at higher concentrations. Elevated concentrations of heavy metals in soil are a serious problem because plants can absorb them, and when these plants are used as feed for domestic animals and

[1] University of Niš, Faculty of Sciences and Mathematics

humans, the harmful effects of heavy metals on animals and humans can occur (Sarwar et al., 2017). The uptake of heavy metals by plants can also be beneficial. Plants that take up a significant amount of heavy metals can be used in the process of phytoremediation and in the phytomining process (Sheoran et al., 2016).

The aim of this work is to examine the uptake of some heavy metals by alfalfa grown on soils artificial contaminated with those metals, both individually and in combination in a pot experiment.

Materials and methods

Setting up a pot experiment

To set up a pot experiment a soil which was not been treated with agrotechnical measures several years ago was used. The soil was taken at a depth of about 20 cm, air dried for about 2 weeks and sieved. Alfalfa clover seeds (*Medicago sativa* L.; Semenacoop, Novi Sad) were purchased in an agricultural pharmacy.

To form the units of the model system, 400 g of the soil was measured in each of 7 pots. The amounts of heavy metals added were equivalent to the concentration found in the test soil except for cadmium (28x higher). The volume of the heavy metal solution used to treat all units of the model system was 100 ml. Appropriate treatments were applied in all 7 pots before sowing clover. The first unit of the model system was treated with deionized water only and served as a reference unit, the second unit of the model system was treated with a mixture of all 5 heavy metals tested, and the next 5 units were treated individually with one metal. After a few days, when the appropriate soil moisture was reached, the clover was sown. Twenty alfalfa seeds were sown in each pot.

Sampling of plant material and soil

100 days after sowing, the plants were taken out and the roots were separated from the above-ground parts. The clover roots were washed first with tap water and then with deionized water to remove the soil. All the plant material was dried in the air and then in a drying oven with ventilation for 8 hours at 70 ℃. The dried plant material was stored in a desiccator until the day of preparation for analysis at ICP-OES. The soil was also dried first in the air and then in a drying oven with ventilation at 95 ℃ for 12 hours.

Preparation of plant material for ICP-OES analysis

Approximately 0.3 g (to four decimal places) of the plant material was measured. 10 ml of conc. HNO_3 (Fisher Chemical) was added to each sample. The contents were left at room temperature for about half an hour and then heated on the hot plate for about 30 minutes until a small volume was reached. The samples were then cooled to room temperature and 2 ml of conc. H_2O_2 (Fisher Chemical) was added to each sample. The samples were again evaporated, cooled, and 5 ml of deionized water was added. The samples were filtered and the filtrates were collected into volumetric vessels of 25 ml (Bargagli et al., 2000).

Preparation of soil samples for ICP-OES analysis: pseudo-total extraction of heavy metals from soil with aqua regia

About 1 g (to four decimal places) of each soil sample was measured. 16 ml of aqua regia was added to each sample, and then the samples were heated in a sand bath at a temperature of about 190 ℃ until a small volume was reached. The samples were cooled after which 6 ml of conc. H_2O_2 (Fisher Chemical) was added. The samples were again cooled and 5 ml of deionized water was added. The samples were filtered and the filtrates were collected into volumetric vessels of 25 ml (Addis and Abebaw, 2017).

Results and discussion

The samples are labeled as follows: the label Ref stands for the reference unit; the symbol of the metal refers to the unit treated with that metal, while the Mix label refers to the treatment of the soil with a mixture of metals. The symbols -S, -R, and -Sh refer to soil, root, and shoot, respectively.

The results of the soil analyses are given in Table 1. Cadmium concentrations in all soil samples are higher than the limit values (The Official Gazette of the Republic of Serbia, 2019). The doping of the soil with single heavy metal solutions leads to exceeding the limit value for Ni, while the doping with a mixture of metals leads to exceeding for Cu and Ni. The differences in the content of the tested metals in the model system units treated with that metal and the metal mixture exist, which points to the potential influence of other metals present except in the case of nickel.

Table 1. Heavy metals in soil (mean ± SD, mg kg^{-1} of dry matter)

Sample	Cd	Cr	Cu	Ni	Pb
Ref.-S	3.70±0.04	25.2±0.7	34±1	27.8±0.2	19.1±0.2
Cd-S	32.2±0.3	26.5±0.2	25.7±0.2	30.1±0.2	20.69±0.08
Cr-S	3.60±0.02	28.0±0.2	20.8±0.2	26.6±0.2	18.38±0.08
Cu-S	3.688±0.005	22.3±0.2	31.5±0.3	26.8±0.1	19.0±0.2
Ni-S	3.67±0.03	21.6±0.2	19.1±0.2	41.10±0.08	18.6±0.2
Pb-S	3.359±0.005	19.3±0.3	18.4±0.3	23.9±0.2	31.4±0.2
Mix-S	36.0±0.2	32.9±0.4	40.7±0.6	40.4±0.3	79.4±0.3
LV*	0.8	100	36	35	85

*limit values of heavy metals in the soil

The concentrations of heavy metals in alfalfa roots are shown in Table 2. Contamination of the soil with heavy metals, both singly and in a mixture, resulted in an increase in the root concentration for all elements. The increased Cd concentration in the soil resulted in noticeable decreased uptake of Ni (38%), Cr (30%), Cu (29%) and only 4% for Pb. The normal concentration of investigated elements in the plant material was exceeded for Cd, Cr and Ni both in the single treatment and in the treatment with a mixture of metals, and for Pb in the case of the mixed treatment (Nagajyoti et al., 2010). According to Alloway (2013) the concentration of Cd in pot units treated with this metal has reached the toxicity threshold (5-30 mg kg^{-1}).

The most abundant metal in the above-ground part of alfalfa is Cu, while Cd is the least abundant (Table 3). As a result of soil contamination with these metals, a significant increase in Cd concentrations in the above-ground part compared to the reference unit is observed. Cadmium concentration in the shoot is higher in the unit of the model system where the soil was treated with a mixture of metals than with Cd alone. All concentrations are in the range of normal i.e. below the toxicity threshold (Alloway, 2013; Nagajyoti et al., 2010).

Table 2. Heavy metals in roots (mean ± SD, mg kg^{-1} of dry matter)

Sample	Cd	Cr	Cu	Ni	Pb
Ref.-R	0.300±0.009	1.84±0.03	5.74±0.09	4.69±0.04	3.72±0.06
Cd-R	5.76±0.02	1.29±0.02	4.09±0.09	2.93±0.05	3.57±0.03
Cr-R	0.333±0.009	4.52±0.05	5.3±0.1	3.68±0.04	3.6±0.2
Cu-R	0.325±0.009	1.69±0.04	9.0±0.2	3.55±0.05	2.6±0.2

Ni-R	0.34±0.02	2.00±0.07	5.16±0.09	6.10±0.02	3.01±0.06
Pb-R	0.3504±0.0000	1.20±0.04	4.32±0.06	2.90±0.03	7.65±0.06
Mix-R	15.58±0.05	4.28±0.02	9.03±0.07	5.73±0.02	16.5±0.1

Table 3. Heavy metals in shoots (mean ± SD, mg kg^{-1} of dry matter)

Sample	Cd	Cr	Cu	Ni	Pb
Ref.-Sh	0.124±0.009	0.27±0.02	7.07±0.03	2.72±0.02	2.0±0.2
Cd-Sh	0.884±0.009	0.21±0.02	6.3±0.1	2.37±0.04	2.24±0.06
Cr-Sh	0.116±0.009	0.249±0.009	4.87±0.09	1.21±0.04	1.01±0.04
Cu-Sh	0.117±0.009	0.16±0.02	6.2±0.2	1.33±0.02	1.41±0.09
Ni-Sh	0.108±0.009	0.133±0.009	6.35±0.07	2.225±0.000	1.20±0.04
Pb-Sh	0.1082±0.0000	0.16±0.02	5.95±0.03	0.691±0.009	1.7±0.1
Mix-Sh	2.80±0.02	0.24±0.02	6.1±0.1	0.99±0.02	1.13±0.08

Root enrichment factor (EFroot) as a ratio of metal concentration in roots of plants growing on polluted and unpolluted soils ranged from 0.62 for Ni to 51.93 for Cd in the soil unit treated with a metal mixture. The highest EF values were for Cd, indicating that alfalfa is capable of absorbing available amounts of Cd in the roots. Translocation factor (TF, shoot/root) is the ratio between the concentration of metals in the above-ground part of the plant and in the root. Values of TF > 1 indicate that the metal is efficiently translocated to the above-ground part of the plant. In most cases, TF values are less than 1, indicating that most of the metals are retained in the alfalfa roots, except for Cu in certain units of the model system.

Conclusion

The contamination of the soil resulted in an increase in the root concentration for all elements. For Cd, Cr, Ni, and Pb the normal concentration in the plant material was exceeded in some root samples. The highest EF_{root} values were for Cd indicating that *Medicago Sativa* L. is able to absorb large amounts of Cd by roots. The TF values (shoot/root) are mostly less than 1, which means that most of the metals are retained in the alfalfa roots, except for Cu in certain units of the model system.

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UPTAKE OF HEAVY METALS BY WHEAT (*Triticum aestivum* L.): POT EXPERIMENT

Denis Mitov[1], Stefan Petrović[1], Nikola Đorđević[1], Jelena Mrmošanin[1], Aleksandra Pavlović[1], Snežana Tošić[1]

Abstract: In the present work, the uptake of some heavy metals by wheat grown on soils artificialcontaminated with Cd, Cr, Cu,Ni and Pb individually and in combination,was studied in a pot experiments. The samples were prepared by wet digestion and analyzed by ICP-OES and the obtained results were discussed based on the values of the obtained concentrations, enrichment factors (EFs) and translocation factors (TFs).

Keywords: wheat, heavy metals, pot experiment, ICP-OES determination

Introduction

Plants need various minerals and other nutrients to grow normally. Much of these minerals and nutrients are taken up from the soil through the roots and then translocated to the above-ground parts of the plant, although certain amounts of nutrients may also be taken up through the leaves. If a plant is growing in soil contaminated with heavy metals, there is a possibility that it will take up some amount of non-essential metals in addition to the essential metals, but also essential metals in greater amounts than the optimal amount, which can lead to a number of negative effects on the plant, such as loss of growth, leaf chlorosis, etc. Accumulation of heavy metals in plants can occur in such quantities that they exceed toxic concentrations for humans and animals.

Wheat (*Triticum aestivum* L.) is one of the most important crops for human consumption worldwide. Worldwide, wheat ranks third in cereal production after rice and corn. Wheat grows in different climates and is one of the most adaptable crops in the world (Miransari and Smith, 2019). In 2021, wheat production reached 778 million tons worldwide. A number of researchers have studied the uptake of heavy metals by wheat: Rezapour et al.(2019), Liu et al.(2009), Nan et al.(2002), Athar and Ahmad (2002). Athar and Ahmad (2002) studied the effects of heavy metals on wheat in a pot experiment and concluded that soil contamination reduces wheat yield. Cadmium had the greatest toxic

[1] University of Niš, Faculty of Sciences and Mathematics

effect, followed by Cu, Ni, Pb, and Cr. As a result of soil contamination with heavy metals, protein content of wheat decreased19.0-71.4%, and metal uptake was directly related to the concentration of heavy metals in soil.

The aim of this study is investigation the uptake of some heavy metals by wheat grown on soils contaminated with heavy metals individually and in combination.

Materials and methods

Setting up a pot-experiment

To set up a pot experiment a soil which was not been treated with agrotechnical measures was used. The soil samples were air dried and sieved. Sevenplastic pots were filled with 400 g of soil each and then treated with heavy metal solutions individually and in combination of all metals. The amounts of heavy metals added were equivalent to the concentration found in the test soil except for cadmium (28x higher). The soil in the pot that served as the reference unit was treated with deionized water only. After a few days, the wheat was sown.

Sampling of plant material and soil

Twenty-five days after the wheat was sown, the plant material and soil were collected and prepared for analysis at ICP-OES. To completely remove the soil from the roots, the roots were washed first with tap water and then with deionized water. The plant material was dried at room temperature for approximately one week and then in a drying oven at 70 °C for 8 hours. The dried plant material was stored in a desiccator until digestion. The soil samples were air dried for two weeks and then sieved. The homogeneous soil samples were dried in the drying oven at 95 °C for 8 hours.

Preparation of plant material for ICP-OES analysis

Approximately 0.3 g of the plant material (root and shoot) was measured to the fourth decimal place and 10 ml of conc. HNO_3 (Fisher Chemical) was added. The contents were left at room temperature for half an hour and then heated on a hot plate for about 30 minutes. 2 ml of conc. H_2O_2(Fisher Chemical) was added to each sample to complete the decomposition of the root samples. Then the samples were filtered and the filtrates were collected into volumetric vessels of 25ml. (Bargagli et al., 2000).

Soil preparation for ICP-OES analysis

From each sample, 1 g of soil is measured.Aqua regia was prepared (mixture of conc. HNO_3 (Fisher Chemical) and conc. HCl (Sigma-Aldrich), volume ratio 1:3) and 16 ml of aqua regia was added to each sample. The soil samples were heated on a sand bath at a temperature of about 190 °C. After one hour, the samples were cooled and 6 ml of conc. H_2O_2(Fisher Chemical) was added. The samples were boiled to a small volume, again cooled and 5 ml of deionized water was added. The samples were filtered and the filtrates were collected intovolumetric vessels of 25ml. (Addis and Abebaw, 2017).

Results and discussion

The samples are labeled as follows: the Ref designation represents the reference unit, the metal designation refers to the unit model system treated with that metal, while the Mix designation represents the treatment of the soil with a mixture of all the metals studied. The designations -S, -R, and -Sh refer to the samples: Soil, Roots, and Shoots, respectively.The results of the soil analysis are given in Table 1.Limit values are exceeded for Cd in all units, for Cu in Cu-S and Mix-S, for Ni in Ni-S and Mix-S and for Pb in Pb-S (The Official Gazette of the Republic of Serbia, 2019).The concentrations of Cd, Ni, and Pb in samples Cd-S, Ni-S, and Pb-S, respectively are highest,while the concentration of Cu is the highest in the Mix-S soil sample compared to all other soil samples.

Table 1.Heavy metals in soil (mean ± SD, mg kg^{-1} of dry matter)

Sample	Cd	Cr	Cu	Ni	Pb
Ref.-S	1.364±0.005	27.1±0.2	22.1±0.2	25.3±0.2	18.1±0.1
Cd-S	35.3±0.2	28.3±0.4	29.1±0.3	26.3±0.4	18.27±0.07
Cr-S	1.32±0.03	47.7±0.9	22.4±0.2	25.7±0.6	18.08±0.04
Cu-S	1.275±0.003	26.2±0.3	39.5±0.5	24.6±0.4	17.43±0.03
Ni-S	1.328±0.008	28.1±0.2	26.87±0.08	46.7±0.3	18.20±0.07
Pb-S	1.270±0.005	26.8±0.3	23.8±0.2	25.6±0.2	86.7±0.1
Mix-S	32.89±0.08	47.5±0.5	49.6±0.3	43.3±0.6	82.4±0.2
LV*	0.8	100	36	35	85

*limit values of heavy metals in the soil

Heavy metal concentrations in wheat roots are shown in Table 2. The potential synergistic effect of the doping metals with respect to the

accumulation of Cd, Cu, and Ni in wheat roots is striking, as confirmed by the maximum concentration of these metals in the Mix-R sample. This probably means that some of the doping metals promote the uptake of the mentioned metals from the soil by the wheat.

Comparison with the normal concentration values in plant material reported in the work of Nagajyoti et al. (2010) shows that value was exceeded for Ni and Cr in all samples and for Cd, Cu and Pb in the samples where the soil was treated with this metal. According to Alloway (2013) the concentrations of all metals except Cu have reached the toxicity threshold in pot units treated with this metal and with mixture of metals.

Table 2.Heavy metals in roots (mean ± SD, mg kg^{-1} of dry matter)

Sample	Cd	Cr	Cu	Ni	Pb
Ref.-R	0.83±0.02	4±0	12.0±0.2	14.15±0.06	8.9±0.3
Cd-R	131±5	4.3±0.4	11±2	20.4±0.2	12±2
Cr-R	0.80±0.04	43.0±0.3	13.2±0.2	40.3±0.2	11.7±0.5
Cu-R	0.76±0.04	3.2±0.2	18.9±0.8	22.3±0.7	15.4±0.4
Ni-R	1.10±0.02	7.68±0.07	12.6±0.2	68.9±0.5	13.7±0.2
Pb-R	0.87±0.04	5.4±0.2	13.3±0.5	17.8±0.2	67.0±0.2
Mix-R	241.1±0.5	14.5±0.2	29.2±0.3	75.5±0.3	53.1±0.6

The potential synergistic effect of the doping metals on the accumulation of the metals Cd and Pb in the wheat shoots is observed, which is confirmed by the maximum average concentrations of these elements in the sample Mix-Sh (Table 3). Comparison with the normal concentration values reported in the work of Nagajyoti et al. (2010)shows that value was exceeded for Ni in all samples, for Cd in the samples where the soil was treated with Cd and metal mixture, for Cr in the reference unit and the unit where the soil was spiked with Cu, while all other concentrations were within the normal values reported.According to Alloway (2013) the concentration of Cd has reached the toxicity threshold (5-30 mg kg^{-1}).Wheat cannot be classified in the group of hyperaccumulator plants for Cd because the concentration of this metal in wheat shoots is less than 100 mg kg^{-1}(Sheoran et al., 2016).

Table 3.Heavy metals in shoots (mean ± SD, mg kg^{-1} of dry matter)

Sample	Cd	Cr	Cu	Ni	Pb
Ref.-Sh	0.242±0.008	2.52±0.02	10.5±0.2	1.52±0.03	2.3±0.2

Cd-Sh	17.18±0.09	0.25±0.03	6.79±0.04	2.03±0.03	2.46±0.06
Cr-Sh	0.265±0.009	0.81±0.02	8.49±0.07	1.42±0.03	2.6±0.2
Cu-Sh	0.29±0.02	1.41±0.04	12.8±0.3	2.15±0.03	2.9±0.2
Ni-Sh	0.39±0.02	0.92±0.03	8.7±0.2	4.54±0.04	4.09±0.07
Pb-Sh	n.d.	0.32±0.03	9.12±0.09	1.81±0.03	4.53±0.04
Mix-Sh	29.2±0.5	0.60±0.07	8.07±0.09	2.85±0.08	5.1±0.4

*n.d.-not detected

All of the metals studied are more strongly retained in the root, as evidenced by translocation factor (TF) values that are less than 1. Cu shows the strongest tendency to migrate into the shoot.Theenrichment factor (EF) was calculated for the shoot of the contaminated units of the pot experiment relative to the shoot of the reference unit of the pot experiment.The EF values for Cr are less than 1 in all samples,for Pb are greater than 1 in all samples, with the highest value for the Mix-Sh sample (EF =2.26). The EF for Cd in the Mix-Sh sample (120.33) is almost twice as high as the EF for the Cd-Sh sample (70.83)which is probably due to the synergistic effect of the metals present. With the exception of the EF factor values for Cr, these factors for all other doping metals are greater than 1 for the units treated with the metal itself.

Conclusion

The results of this study indicate the potential synergistic effect of doping metals on the acumulation of Cd, Cu, and Ni in wheat roots and for Cd and Pb, in wheat shoots. For Cd, Cr, Ni, andPb, an decreased distribution from root to shoot was generally observed in all samples. Although the EF for Cd is greater than 1 in all wheat shoots, wheat cannot be classified in the group of hyperaccumulator plants for cadmium.

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A MATHEMATICAL MODEL FOR CONTROLLING THE ACIDITY OF A SOLUTION IN A BIOREACTOR OF THE ARTIFICIAL GIT OF POULTRY

Alexander D. Lukyanov[1], Danila Y. Donskoy[1],
Vladimir Filipović[2], Tamara B. Asten[1]

Abstract: Reactions of neutralization occur with significant nonlinearity, which determines the control algorithms in the mode of periodic dosing of a volume of acid or alkali. Such regulation may have little effectiveness due to the fluctuation or impossibility of regulation due to insufficient or excessive concentration. This article precisely regulates the hydrogen index in mini-bioreactors and proposes the use of a digital model of the acidity management system for the selection of concentrations of topped-up solutions, determination of the regulation methodology, and improvement of accuracy pH regulation in an in vitro mini-model of the gastrointestinal tract (GIT) of a static type.

Keywords: mathematical model, bioreactor, GIT, digital model

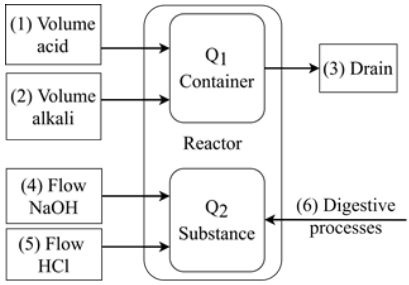
Introduction

In this paper, we consider the solution to a particular problem of mathematical modeling of the pH regulation process for an in vitro mini-model of the gastrointestinal tract (gastrointestinal tract) of a static type. The importance of precise control of the acidity of the chyme is determined by rather strict requirements for the conditions of in vitro experiments. Ensuring plausible conditions for the course of digestive processes, as well as the requirements for repeatability of experiments, require automatic pH control in the bioreactor with an accuracy not worse than ±0.1 units. At the same time, the pH range can be from slightly alkaline (7.9 pH, oral cavity) up to strongly acidic (1.3 pH, stomach).

[1] Don State Technical University, Ploshchad' Gagarina 1, Rostov-on-Don, Russian Federation (alexlukjanov1998@gmail.com)
[2] University of Novi Sad, Faculty of Technology Novi Sad, Bul. cara Lazara 1, Novi Sad, Serbia

Materials and methods

The reactor as a physical object simultaneously acts as a storage of the amount of liquid (Q1) and storage of the number of hydrogen ions (Q2) (Graph 1). This is a container in which a controlled digestion process takes place, accompanied by a change in pH and a change in fluid volume (Alegría et al., 2015; Mackieet al., 2015; Steinway et al., 2020).



Graph 1. Block diagram of the model

To simulate the neutralization reaction, we will use the method of accumulators and flows. The method is based on the use of fundamental or local conservation laws (accumulators) and mathematical description of patterns that change the amount of accumulated feature (flows).

The methodology of the experiment is as follows (Alegría et al, 2015; Mackieet al., 2015). The mathematical model takes into account the characteristics of a real system, such as:

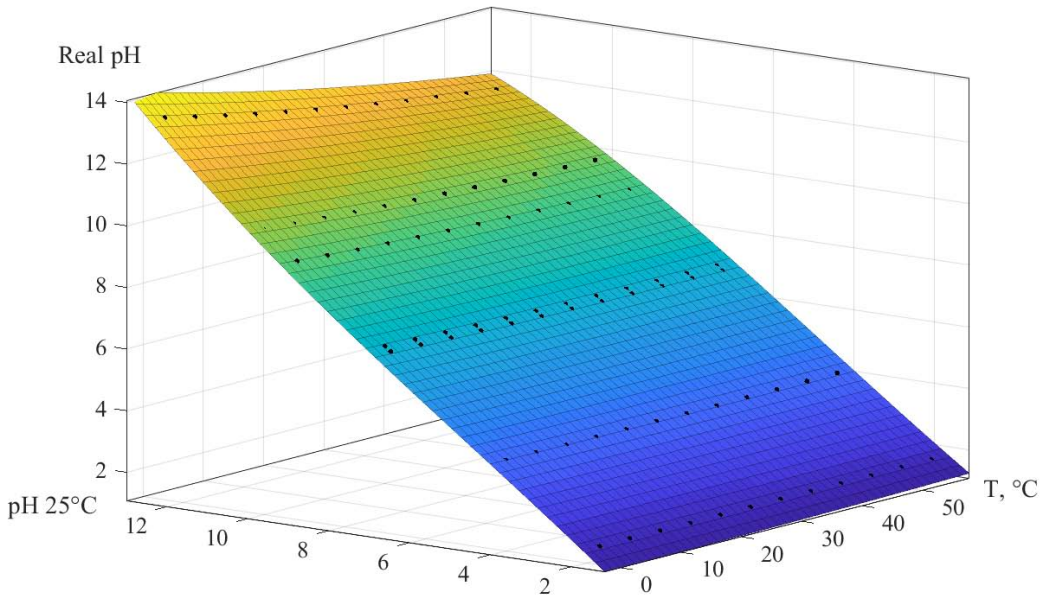
- Set acidity;
- Reactor parameters (base area and height of the bioreactor);
- Concentrations of added substances (acids and alkalis);
- Initial concentration of the solution;
- Initial volume of the solution;
- The temperature of the solution throughout the experiment;
- Discreteness of the control process.

Results and discussion

The system of differential equations describing the dynamics of changes in the volume and concentration of the solution in the bioreactor is presented in the Eq. 1 (Steinway et al., 2020):

$$\begin{cases} \dfrac{dh}{dt} = \dfrac{1}{S}(G_1 + G_2 - G_3) \\ \dfrac{dC}{dt} = \dfrac{1}{S \cdot h}\left(G_1(C_{NaOH} - C) + G_2(C - C_{HCl})\right). \end{cases} \quad (1)$$

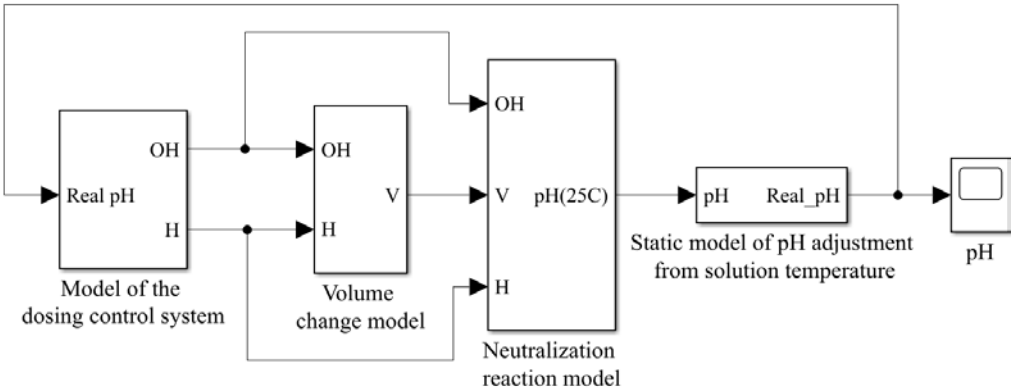
Thus, the acidity of the solution was obtained under normal conditions, that is, at 25 ℃. But the contents of the bioreactor are not always in normal conditions. For example, to create an artificial environment characteristic of the in vivo environment of the gastrointestinal tract of poultry, a temperature of 42℃is maintained in the reactor.



Graph 2. A surface describing the dependence of pH on temperature

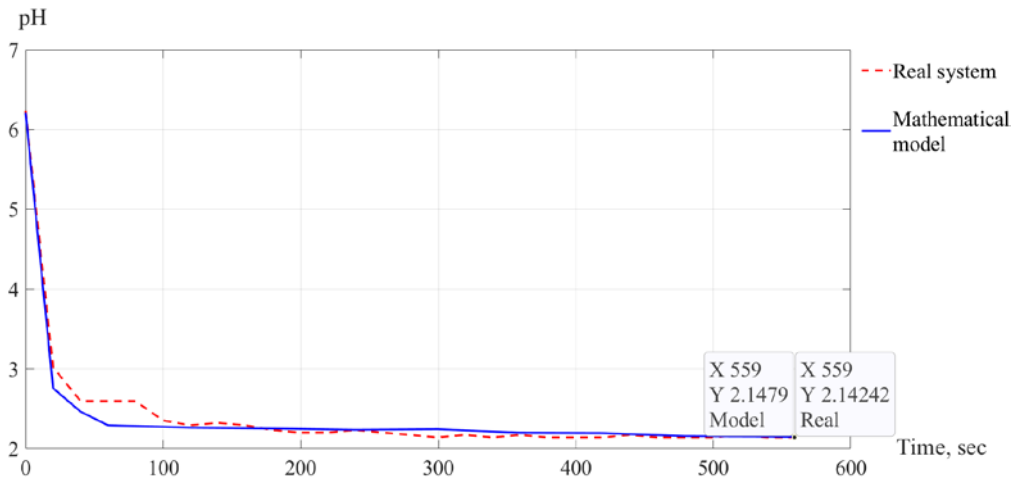
Based on experimentally and based on available research data, a static model was constructed, it was obtained by polynomial approximation by the least squares method. A visual representation of the pH dependence on temperature is shown in Graph 2.

To determine the transients in the system, we will build a mathematical model in the Matlab Simulink application package, see Graph 3:



Graph 3. The general structure of the mathematical model in Matlab Simulink

One of the experiments was carried out at a given acidity of the solution in the range of 2.15 ± 0.05 pH. The acidity of the initial solution is 6.2 pH. The temperature of the solution in the reactor: is 22.25 ℃. Solutions of 0.1 mol L^{-1} NaOH and 0.1 mol L^{-1} HCl were used to change the acidity. The dosing algorithm consisted of discrete proportional regulation.



Graph 4. Transient characteristics of a mathematical model and a real system when regulating from pH 6.2 to 2.15

Conclusion

The paper presents a mathematical model of the pH control process intended for use in the bioreactor pH control system. The model showed good agreement with the experimental data and can be further used as a reference model in control systems to improve control accuracy.

Acknowledgement

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MICROBIOLOGICAL COMPLEX FOR MODELING PROCESSES IN THE GASTROINTESTINAL TRACT OF ANIMALS

Alexandr D. Lukyanov.[1], Danila Y. Donskoy[1], Miroslav A. Vernezi[1], Maria S. Mazanko[1], Tatiana S. Onoyko[1]

Abstract: *In vivo* modeling of digestive processes is not a standard mathematical modeling task, taking advanced technologies of several sciences. This article will consider a microbiological complex for modeling the processes occurring in the gastrointestinal tract of animals. The system belongs to mini-models with a working volume of up to 400 ml, the simulation of the dynamics of the mixing of the chyme is static, which significantly simplifies the methodology of conducting experiments, but imposes difficulties on the stages of pretreatment of food matrices. The following characteristics are achieved: reactor volume from 50 to 100 mL; a pH accuracy of ± 0.1; temperature accuracy of ± 0.063 °C.

Keywords: modeling, microbiological complex, mini-model, *in vitro*

Introduction

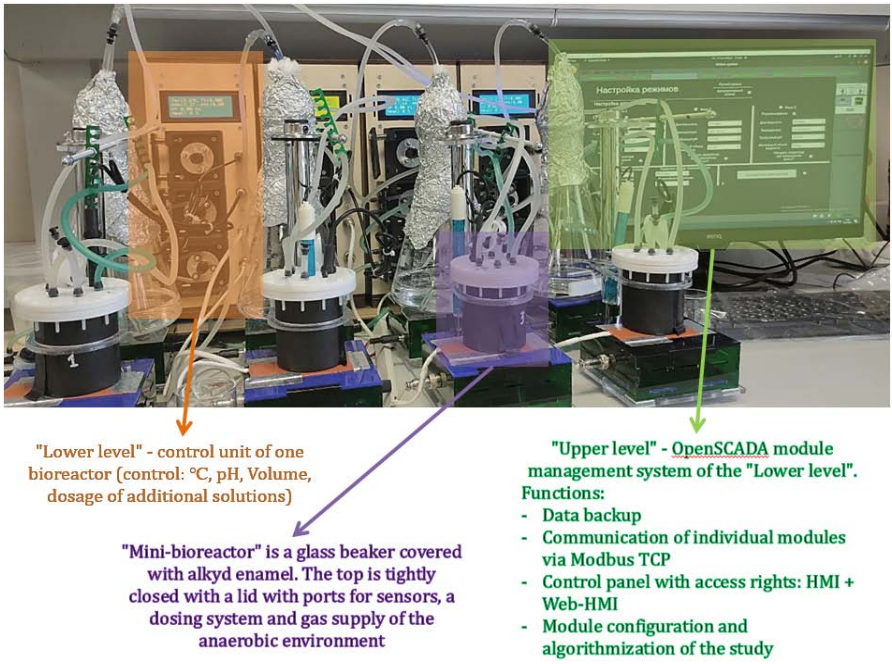
Modern methods of mathematical modeling of dynamic processes already make it possible to create primitive artificial systems for conducting in vitro studies relative to *in vivo* processes. Since the 80s, research has been undertaken aimed at modeling digestive processes in the human gastrointestinal tract. Since 2019, based at the Don State Technical University, a joint team of biologists and veterinarians has been developing a static-type microbiological complex for complex modeling of processes in the gastrointestinal tract of animals (Alegría et al., 2015; Chistyakov et al., 2020).

Materials and methods

The microbiological complex integrates several levels of equipment responsible for managing certain nodes or a network of nodes. The basic control algorithms are based on the experimental results of SHIME, DIGI, and long-term experience in the development of *in vitro* models of the gastrointestinal tract. The system can be divided into 3 levels (Chistyakov et al., 2020; Graph 1).

[1]Don State Technical University, Ploshchad' Gagarina 1, Rostov-on-Don, Russian Federation (alexlukjanov1998@gmail.com)

The "upper level" is the main computing and controlling node, it is based on an OpenSCADA system installed on a PC or an embedded microcomputer. In different modifications of the microbiological complex, you can find different configurations of this level. The "upper level" implements packet data transmission and provides distributed communication of the "Lower level" nodes. This allows you to flexibly orient the system and interact with it in different scenarios. For example, an operator can use only a part of the "Lower Level" blocks and work with them as with independent unrelated nodes. This is convenient if it is necessary to analyze a batch of samples with different properties in the same type of environment of mini-bioreactors.



Graph 1. The microbiological complex of artificial modeling of animal digestive tract processes

The "lower level" are modules for monitoring and controlling parameters in bioreactors based on a microcontroller system with sensors and actuators. The module controls the temperature, the intensity of mixing of the chyme, the hydrogen index, and the concentration of solutions by maintaining a single working volume throughout the experiment.

A "mini-bioreactor" is a device designed for the cultivation of microorganisms, it is in it that the environmental parameters controlled by the previously described nodes are maintained. The bioreactor consists of a chemically neutral container, a sealed lid with ports for sensors, a dosing system, and a gas supply. The gas supply system is controlled directly by the operator and is necessary for conducting studies of microorganisms in an anaerobic environment.

Results and discussion

The developed microbiological complex implements dynamic temperature control of the contents of bioreactors in the range from +10 to +50 ℃ with an accuracy of ± 0.0625 ℃. This is possible by an adapted algorithm that takes into account the parameters of the bioreactor and the volume of its contents. The required hydrogen index of the solution in the bioreactor is provided with an accuracy of ± 0.1 pH. pH control is based on the analysis of a mathematical model. Accurate dosing of acids and bases requires a preliminary calculation of the volume, and concentration of solutions, as well as accurate readings of the electrodes. Therefore, all sensors are calibrated individually in advance, both by the temperature change in the readings of the electrode itself and by the temperature fluctuations in the acidity of the solutions in which they are located (Mackie et al., 2015).

As a result of research and development, the following characteristics of the microbiological complex were obtained:

- it is possible to use two standard sizes of the reactor, with a useful volume from 200 to 300 mL and from 50 to 100 mL;
- it is possible to simultaneously manage up to 32 channels (independent or interconnected bioreactors);
- implemented dynamic temperature control of the content of bioreactors in the range from +10 to +50 ℃ with an accuracy of ±0.063 ℃ and transient process duration of ~300 s. This is possible due to an adapted algorithm that takes into account the parameters of the bioreactor and the volume of its contents.
- regulation of the pH value of the solution in the bioreactor is provided with an accuracy of ±0.1 pH. The control range is determined by the molarity of the acid and alkali solutions used. pH control is based on the analysis of a mathematical model. Accurate dosing of acids and bases requires a preliminary calculation of the volume, and

concentration of solutions, as well as accurate readings of the electrodes. Therefore, all sensors are individually calibrated in advance, both by temperature changes in the readings of the electrode itself and by temperature fluctuations in the acidity of the solutions in which they are located;

- the dosing accuracy of peristaltic pumps is 0.2 %, which allows for long-term experiments on the study of digestion processes with periodic topping up and selection of small volumes of nutrient medium. Within 5 days in the mode of topping up/selection every 30 min. 2 ml, the change in the volume of liquid in bioreactors did not exceed ± 10 %;
- implemented the maintenance of an inert atmosphere inside the bioreactors with an excess pressure of about 500 – 1000 Pa, which excludes the penetration of oxygen into the atmosphere.

From the point of view of the capabilities of the control system, the time of continuous operation of the system is not limited. The system was tested for 7 days in a sterile environment. Sterility was not violated, and clouding of the medium was not registered.

System tests were carried out using the following strains of microorganisms and culture media:

- *Lactobacillus*, MRS (LenReaktiv)
- *Bifidobacterium*, *Bifidobacterium* Isolation Medium (HiMedia)
- *Enterococcus Bifidobacteria* Isolation Medium (HiMedia)
- *E.coli*, Endo (HiMedia)
- *Proteus*, Endo (HiMedia)
- *Citrobacter, Enterobacter*, Endo (HiMedia)
- *Bacillus* LB medium

When simulating the digestion conditions of the chicken rectum: temperature 42 ℃, pH 8.5 adding/withdrawing 2 mL of the medium every 30 minutes for 60 hours, all bacteria successfully developed to concentrations of $1.4 \times 10^7 – 3.8 \times 10^8$ which corresponds to the development of bacteria under similar conditions in flasks.

Thus, the obtained characteristics of the setup meet the requirements for *in vitro* microbiological and physiological experiments.

Conclusion

Thus, the developed microbiological complex provides 2 types of research. Sequential – where each module of the "Lower Level" represents a specific organ of the digestive tract of an animal and its contents are transferred sequentially through all reactors. Parallel – where each module, as an independent unit, carries out continuous cultivation of microorganisms, which makes it possible to analyze in detail the parameters of the experiment after the study for their comparison with each other or with *in vivo* characteristics.

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