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14–15 March 2024

Faculty of Agronomy in Čačak, University of Kragujevac, Serbia

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PREFACE

"From the mouth of my immortal teacher Pasteur, I heard these words:
It is true that science is international, but every scientist must be a man who in his scientific work is warmed by love for the people from which he sprang and to whom he owes all his strength".

Prof. Dr. Milan Jovanović Batut (1847-1940)"
The first dean of the Faculty of Medicine in Belgrade

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, 29y traditionally organizes the Symposium on Biotechnology (SYMBIOTECH) every year. The main goal 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. We work tirelessly in pursuit of excellence.

At the 2nd International Symposium on Biotechnology, a total of 80 papers were presented in the 7 sections: Field, Vegetable and Forage Crops, Pomology and Viticulture, Livestock Production, Plant Protection, Food Safety and the Environment, Food Technology, Nutritionism, 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 traditional financial support and patronage of SYMBIOTECH24. We thank companies, entrepreneurs, stakeholders and all long-time friends of the Faculty of Agriculture for their material and organizational support.

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CHEMOMETRICS IN AGRI-FOOD BUSINESS AND SUSTAINABILITY

Predrag Putnik[1], Daniela Šojić Merkulov[2], Branimir Pavlić[3], Branko Velebit[4] and Danijela Bursać Kovačević[5],

Abstract: This paper reviews the juncture of chemometrics and sustainability in the agri-food industry. Chemometrics, as a powerful analytical set of tools, has a crucial role in promoting sustainability by improving resource efficiency, reducing waste, and enhancing the overall environmental footprint of agri-food production and processing. Through various applications, this paper reports how chemometric contributes to realizing sustainable practices while keeping quality standards in the agri-food business.

Keywords: chemometrics; PCA factor analysis; waste management for sustainability; mathematical modeling for optimizing/predicting; research methodology food science

Introduction

The agri-food industry faces multidimensional challenges, including safeguarding food quality, safety, and sustainability (Granato, Putnik et al. 2018, Kovačević and Putnik 2022). In this context, chemometric plays an essential part by providing powerful tools for data analysis, process optimization, and quality assessment. To put it differently, it is crucial for the field of food science by providing powerful tools for complex data analysis (Putnik, Šojić Merkulov et al. 2023). Chemometrics commonly employs statistical and mathematical methods to analyze intricate data sets in the context of food science and technology that are usually obtained from "wet chemistry"

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or modern instrumental techniques as spectrophotometry, chromatography, and other analytical methods (Putnik, Granato et al. 2019). Accordingly, it allows food experts to extract concrete conclusions from data, contributing to improvements in overall food quality, safety, and production (Roberts and Cozzolino 2016). The aim of this paper explores the intersection of chemometrics, agri-food business, and sustainability as shown on Figure 1.

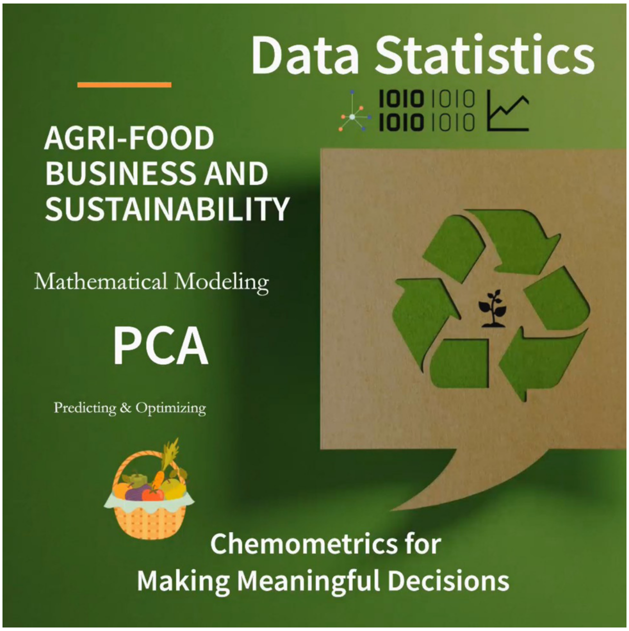

Figure 1. Application of chemometrics in agri-food business and sustainability

Materials and methods

This work reviews references from Web of Science, PubMed, Scopus, and other sources that provide scientific information. Furthermore, several search engines such as Google.com, Ask.com, Bing.com, and other, provided terms related to the topic of the study. The manuscript is intended to provide different applications and contributions of chemometric (and related research methodology and statistical tools) and its roles for reaching sustainable practices in modern food production/agri-food business, while retaining usual quality standards (Croarkin 2013). It is important to note that this brief review is by no means all-inclusive in listing all aspects of applied chemometrics in agri-food business, rather it should be considered as a good starting point for further research for widening personal (professional) knowledge about vast amount of available information related to research methodology, so that entire field of agri-food generates as much as possible high-quality data.

Results and discussion

Probably for more than three decades, food scientists have worked with immense amounts of data from numerous measuring devices (e.g. instrumental and sensory data) (Lazarević, Putnik et al. 2022). The incorporation of multilateral analytical procedures for complex food assessments and productions, requires corresponding multifactorial statistical tools that are able to simultaneously analyze more than one independent variable and effects they have on a one (or more) dependent variables. The important argument here is "simultaneous comparison," that has tendency to decrease type I errors, otherwise known to falsely displays effects and significances that do not really exist (Dumancas, Ramasahayam et al. 2015). Thus, chemometrics has become a vital part of routine in food analysis. It can be applied for datasets that include variables about food chemistry, nutritive contents, authenticity, aromatic profiling, food safety etc. (Jurica, Brčić Karačonji et al. 2021, Bebek Markovinović, Stulić et al. 2023, Bebek Markovinović, Stulić et al. 2024). In general, chemometrics refers to the quantitative analysis of foods using multivariate statistical data analysis. It helps classify and model data, seek underlaying relations among them, while employing techniques as principal component analysis (Wold, Esbensen et al. 1987), cluster analysis (Aldenderfer and Blashfield 1984), multivariate analysis of variance, mathematical modeling (regression analysis), and many other.

Assimilating chemometrics into agri-food practices contributes to sustainability by evaluating advanced (functional) food technologies and raw materials (Bebek Markovinović, Brčić Karačonji et al. 2022, Bebek Markovinović, Putnik et al. 2022b, Bebek Markovinović, Putnik et al. 2022c), optimizing processes (Škegro, Putnik et al. 2021, Bebek Markovinović, Putnik et al. 2022a), minimizing waste (Bognár, Putnik et al. 2022a, Bognár, Jovanović et al. 2024), enhancing product quality (Bebek Markovinović, Putnik et al. 2023), assessing environmental toxicity (Bognár, Putnik et al. 2022b, Bognár, Jovanović et al. 2024), hence testifying that chemometrics aligns quite well with ecological resilience. Next, statistical tools and approaches from chemometrics can be very useful in the role of business intelligence in the food industry, focusing on how data analytics and insights drive decision-making, optimize operations, and enhance competitiveness (Putnik, Šojić Merkulov et al. 2023). With the arrival of digital technologies and the spread of data sources, business intelligence has emerged as a critical tool for food companies to gain actionable insights, improve efficiency, effectiveness and meet evolving consumer demands.

It is interesting to observe connection of chemometrics with business intelligence and how it relates with food quality 4.0 on a level of a product (Djekić, Velebit et al. 2023). So to analyze the trends and existing levels of food quality 4.0 in literature in our previous work we conducted a bibliometric overview of publications by applying text mining to identify the main research streams. It consisted of three activities: (i) data collection, (ii) data processing and cleaning, (iii) visualization and interpretation. The input data (abstract, title, and keywords) were extracted from academic papers indexed in the Web of Science with the search term "food quality" + "industry 4.0." The search was set to include only articles / reviews papers (excluding book chapters and proceedings). The time period spanned from 2013-2022 (10 years). The analysis revealed four distinct clusters: (i) quality characteristics; (ii) Internet of Things; (iii) Industry 4.0 and (iv) food safety. The first cluster had a strong link between quality and different quality attributes, such as physicochemical or functional properties (Djekić, Velebit et al. 2023). The second cluster highlighted the connection of Industry 4.0 with machine learning in food sustainability and agriculture. The third one focused on various Internet of Things technologies such as blockchain, artificial intelligence, and Big Data. Finally, the food safety cluster was related to the food supply chain and various predictive and traceability tools (as chemometrics). From this analysis, it was clear that food scientists use chemometrics mostly for food safety within the Food Quality 4.0 for improvements of food products.

Assessments of chemometrics in agri-food business and sustainability often incorporate techniques that include design-of-experiment methodology (Croarkin 2013, Dziurkowska and Wesolowski 2015), multivariate exploratory analyses, and regression modeling (Hidalgo and Goodman 2013). As mentioned earlier, these tools help out interpreting complex datasets obtained from the advanced analytical methods assisting in assessing food quality, ensuring authenticity, and optimizing manufacturing processes. For instance, in microbiology, chemometrics can assist in understanding the impacts of microbial parameters on food quality and safety. Researchers' usage of multivariate statistical tools to relate bioactive components with functional properties and microbial behavior is another example how multifactorial methods can be useful (Putnik 2023). Effects of processing can be also determined by the chemometrics, i.e. how various advanced processing variables affect the chemical composition of foods (Bebek Markovinović, Brdar et al. 2024). By analyzing complex and multivariate data, this provides insights into how processing methods influence food quality and safety (Granato, Putnik et al. 2018). Furthermore, governmental bodies and industries in practice can employ chemometric tools for monitoring of food quality, raw materials, and production processes. Researchers consider both the pros and cons of different chemometric tools to choose the most appropriate statistical approach for their specific studies. Hence, chemometrics serves as a valuable aid when addressing multifaceted real-life problems in food science. Its applications extend from authentication to microbiology and process optimization (Granato, Putnik et al. 2018).

Mathematical modelling is multivariate tool from the chemometric portfolio of techniques that can be used for above purpose. It is a powerful tool in food science, enabling researchers and industry professionals to understand complex phenomena, optimize processes, and develop innovative products by building mathematical equations (Croarkin 2013). Its diverse applications in food science, encompasses areas such as food engineering, microbiology, nutrition, and sensory analysis. Through computational simulations, predictive modeling, and optimization techniques, mathematical models provide valuable insights into food properties, behavior, and various interactions. Following in the text key principles, methodologies, and applications of mathematical modeling in food science, will be examined and highlighted with its role in driving sustainable practices, improving food safety, and meeting consumer demands.

In practice, optimizations/predictions give various advantages for industrial purposes, as giving the edge over competitors (e.g. efficient production of food, optimal utilization of the market, identify customers from the available pool, prediction of customer acceptance, various aspects of business intelligence, etc.). It involves the construction of mathematical representations (equations) that capture essential features and behaviors of the system being studied. These models are used to analyze, predict, and understand the behavior of the system under various conditions and its extrapolations for different combinations of parameters. For building useful mathematical equations some form of multivariate regression analysis is employed with all potentially related factors (Hidalgo and Goodman 2013). Importance of each contributor in the equation must be evaluated by statistical significance while insignificant variables must be removed from the equation (Croarkin 2013).

Some trends related to chemometrics in food science include food authentication in verifying the genuineness of food products. By analyzing chemical markers, it helps identify the geographical origin, farming systems, and potential adulteration of food products (Jurica, Brčić Karačonji et al. 2021). Techniques such as pattern recognition, including principal component analysis (PCA) and cluster analysis, are commonly used for this purpose (Wold, Esbensen et al. 1987).

The term factor analysis is referred to group of different but related statistical methods. From those, the most popular method applied to the agri-food business is named PCA that employs reduction of large set of variables to create as less as possible dimensions that can account for the larger portion of variance in the dataset (relaying on linear correlation). In PCA original variables are transformed in smaller set of linear combinations with use of the entire variance from dataset for obtaining empirical summary of the data set (Pallant 2020). It is important that analyzed data have sufficient proportion of variance that might be caused by underlying factors (Tabachnick and Fidell 2007). One of the prerequisites for conducting PCA (or any type of factor analysis) is Kaiser-Meyer-Olkin measure of sampling adequacy (KMO) and Bartlett's test of sphericity. KMO is suggested to be at minimum of 0.6 or more (some sources set this minimum threshold to 0.7), and Bartlett's test should be significant at $p \leq 0.05$. Regarding the sample size for PCA, the upper limit was not detected in the literature, while recommendations for the lower limit are suggested to 10:1 ratio, as in 10 cases per single item in the PCA. Others suggested that 5 cases per item is adequate for the most situations (Mundfrom, Shaw et al. 2005). Recommended software for performing PCA is IBM SPSS Statistics or SAS that are commercially available and popular among scientific community, while another good and freely available software is R. However, R might be difficult for novice to use it with all of the need for typing and programing.

Conclusion

The agri-food industry is faced with multifaceted challenges, including ensuring food quality, safety, and sustainability. It certainly does seem true that chemometrics plays a fundamental role in providing powerful tools for data analysis, process optimization, and quality assessment. In recent years, consumers globally have shown an increased interest for high levels of food quality. Accordingly, stakeholders aim to balance food production, sensory quality, nutritional value, and health properties while ensuring sustainability in manufacturing. Chemometrics encompasses techniques like design-of-experiment methodology, multivariate exploratory analyses, and regression modeling. These tools help interpret complex datasets arising from state-of-the-art analytical methods. Referenced examples demonstrated how chemometrics may assist in food production and processing while contributing to food production sustainability. By mathematical modeling chemometrics focuses on optimizing industrial processes, minimizes waste with enhancing product quality and safety. Hence, chemometric data analysis aligns with environmental sensibility and contributes to ecological resilience allowing academia, industry, and regulatory bodies to drive further sustainable practices in agri-food business. It is important to note that modeling is intended for prediction or optimization with some mathematical equation. While PCA is intended for finding the structure in data, so that analyst has easier time in dealing with large datasets for producing meaningful conclusions. Both statistical methods have wide applications in food industry and elsewhere. In conclusion, chemometrics serves as a bridge between analytical science, food quality, and sustainable practices. Its wider adoption can lead to safer, healthier, and more environmentally conscious agri-food systems, so researchers should continue exploring innovative applications of chemometrics in the future.

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SOIL SUITABILITY FOR AGRICULTURAL PRODUCTION IN THE AREA OF PROGAR (SURČIN MUNICIPALITY, BELGRADE)

Aleksandra Stanojković-Sebić[1], Darko Jaramaz[1], Zoran Dinić[1], Jelena Maksimović[1], Aleksandar Stanojković[2], Radmila Pivić[1]

Abstract: The aim of the paper was to conduct the research related to the examination of basic chemical parameters and texture class of agricultural soil, up to depth of 30 cm, in the area of Progar (Surčin Municipality) in order to improve them from the aspect of chemical reclamation and assessment of suitability for agricultural production. The results obtained indicate that the examined soils are mostly suitable for intensive field production, mainly wheat, maize and sunflower. Accordingly, general recommendations for fertilisers and liming materials application, are given for the mentioned plants.

Keywords: Progar, Surčin, chemical parameters, textural class, field production

Introduction

The Municipality of Surčin is located northwest of Belgrade (Republic of Serbia) and covers an area of 288 km^2. It is mostly characterized by agricultural-processing sector, where the share of agricultural soil in 2012 was about 72.6%. The quality of these soils is very different. The most fertile areas are in Surčin around the airport, while the lower classes are in the settlements of Boljevci, Bečmen, Progar and Petrovčić. The agricultural soil of the studied Progar area in 2012 was about 2426 ha. These soils mainly consist of arable soils intended for field production (Janošević et al., 2012). During the field survey it was observed that certain soils, intended for agricultural production, are marginal, in the sense of their limitations. Thus, the position of Surčin itself, which gravitates to the capital city, requires the need to try to enable each surface and turn it into a production area pursuant to appropriate application of agro-technical measures, mainly mineral fertilisers.

The aim of the paper was to conduct the research related to the examination of basic chemical parameters and texture class of agricultural soil in the area of Progar (Surčin Municipality) in order to improve them from the aspect of chemical reclamation and assessment of suitability for agricultural production.

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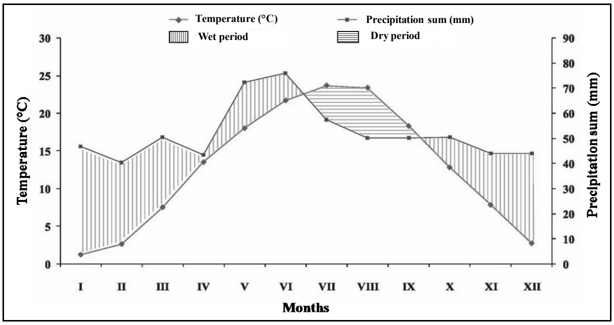
Materials and methods

The trial was conducted in the area of Cadastral Municipality (CM) Progar, Surčin Municipality, Belgrade, Republic of Serbia (grid ref.: 44°42′N, 20°09′E).

Climate data for a series of 16 years of observation (period 2003-2018) were taken from the available meteorological yearbooks of the Republic Hydrometeorological Institute of the Republic of Serbia, and processed graphically. Data from the meteorological station Surčin (location: 44°49' N, 20°17' E, altitude 99 m above sea level), which covers with data the narrower and wider area of research, were used. The relation between wet and dry periods during 2003-2018 is presented using climate diagram according to Walter (Graph 1). The diagram was obtained on the basis of average mean monthly values of air temperature and average mean monthly precipitation heights in the ratio 3:1. Diagram shows that the dry period occurs from the end of June and lasts until the last decade of September. This precipitation regime does not positively affect either vegetable or fruit production, but are most favorable for crop production.



Graph 1. Climate diagram - ratio of dry and wet periods for 16 years of observation

Field work included taking composite soil samples with a probe at pre-determined locations in a disturbed state from a depth of 0-30 cm, then, packaging, transport and storage of samples, according to standard method (ISO 18400-105:2017). Soil samples (112 in total) were taken during July and August 2019 on 24 locations (cadastral parcels, CP) within CM Progar.

Based on the available satellite images and the pedological map of Institute of Soil Science, scale R=1:50.000, soil types (Eutric Cambisol and Fluvisol) and the observation places where sampling was performed, were located (Figure 1).

The soil samples preparation and analysis were performed using standard method (SRPS ISO 11464:2004). Soil acidity (pH in H_2O and in 1M KCl), $CaCO_3$ and total N content were analysed according to standard metods (SRPS ISO

10390:2007; SRPS ISO 10693:2005; SRPS ISO 13878:2005, 2005). Available P and K were analysed by AL-method, spectrophotometrically and flamephotometrically, respectively. Humus content was determined using Kotzman method (Džamić et al., 1996). Textural soil class was obtained using the International Union of Soil Science (IUSS) texture triangle (Murano et al., 2015), based on the share of clay, sand, and silt fractions in soil, respectively. The results are presented using Microsoft Office Excel 2007 statistical and mathematical program, where the following values were calculated: mean (AVR), STDEV and frequency. GIS software was used as a platform for geostatistical data analysis in spatial data processing.

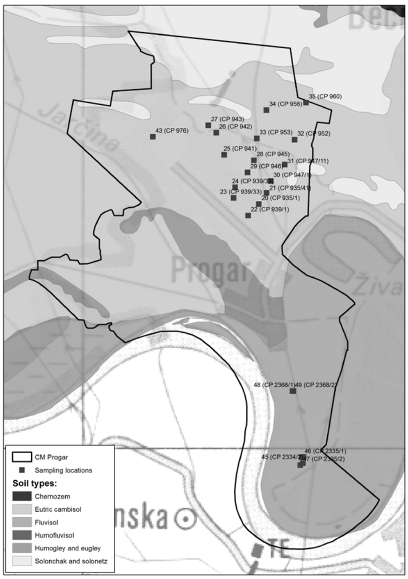


Figure 1. Pedological map and sampling locations of CM Progar study area (source: Institute of Soil Science, 2019)

Results and discussion

Table 1 displays the values of the basic chemical soil parameters in analysed soils of CM Progar.

The results showed that the tested Eutric Cambisol soils are characterized by the following features: strongly to slightly acid reaction (pH in 1M KCl), strongly to neutral reaction (pH in H_2O), with almost all samples without $CaCO_3$; very low to highly provided with available P, low to very highly provided with available K, insufficiently to well provided with total N, and low to highly provided with humus

(Džamić et al., 1996). The results of $CaCO_3$ contents are according to the obtained pH values, where low pH values indicate an adequate use of limestone. The low P content may be a consequence of many things, such as the presence of Ca in alkaline soils, but also of reduced P nutrition (Popović, 1989).

Table 1. Basic chemical properties of the studied area soil samples

CP	Basic chemical parameters of soil fertility (mean±stdev)						
	pH		P_2O_5	K_2O	N (%)	Humus	$CaCO_3$
	1M KCl	H_2O	(mg100g^{-1})	(mg100g^{-1})		(%)	(%)
Soil type: Eutric Cambisol							
935/1	4.7±0.32	5.9±0.32	12.9±12.73	25.0±4.25	0.149±0.02	2.4±0.07	-
935/41	5.8±0.14	6.9±0.07	12.7±2.93	22.5±3.08	0.150±0.02	2.3±0.38	0.43±0.61
939/1	5.2±0.00	6.4±0.00	33.8±0.00	40.3±0.00	0.170±0.00	2.2±0.00	-
939/33	4.3±0.00	5.0±0.00	5.9±0.00	31.1±0.00	0.134±0.00	2.9±0.00	-
939/39	4.3±0.00	5.2±0.00	6.8±0.00	33.4±0.00	0.178±0.00	3.4±0.00	-
941	4.3±0.29	5.2±0.20	6.7±2.80	18.9±2.77	0.148±0.00	2.6±0.14	-
942	4.7±0.00	5.8±0.00	1.7±0.00	9.4±0.00	0.284±0.00	6.2±0.00	-
943	4.6±0.44	5.7±0.40	16.5±14.91	39.3±27.74	0.158±0.02	2.7±0.35	-
945	4.6±0.43	5.6±0.56	4.4±2.89	16.7±3.17	0.125±0.03	2.6±0.83	-
946	4.3±0.00	5.2±0.00	2.1±0.00	16.3±0.00	0.109±0.00	1.9±0.00	-
947/1	4.6±0.33	5.7±0.35	9.8±7.65	24.1±5.34	0.138±0.01	2.2±0.20	-
947/11	4.3±0.32	5.4±0.44	22.9±11.06	44.2±23.83	0.193±0.04	2.9±0.43	-
952	3.9±0.07	4.9±0.07	13.4±1.81	23.0±4.42	0.155±0.01	2.7±0.09	-
953	4.5±0.63	5.5±0.73	6.1±3.67	23.3±4.20	0.130±0.01	2.3±0.25	0.14±0.38
956	4.7±0.66	5.7±0.67	10.9±11.14	38.5±27.08	0.163±0.03	2.9±0.69	0.23±1.19
960	3.9±0.00	5.0±0.00	3.2±0.00	14.2±0.00	0.156±0.00	2.8±0.00	-
962	4.9±0.93	5.9±0.89	8.7±7.79	22.7±15.56	0.147±0.02	2.6±0.50	0.14±0.62
970	4.3±0.00	5.2±0.00	1.7±0.00	18.5±0.00	0.222±0.00	3.7±0.00	-
976	4.2±0.10	5.3±0.10	6.1±1.95	14.9±2.05	0.120±0.01	2.5±0.16	-
Range	3.9-5.8	5.0-6.9	1.7-33.8	9.4-44.2	0.109-0.284	1.9-6.2	-
Soil type: Fluvisol							
2334/2	6.7±0.00	7.6±0.00	16.3±0.00	15.8±0.00	0.200±0.00	2.3±0.00	7.78±0.00
2335/1	7.1±0.00	8.0±0.00	9.7±0.00	13.9±0.00	0.179±0.00	2.9±0.00	4.32±0.00
2335/2	7.0±0.00	8.2±0.00	5.7±0.00	15.7±0.00	0.196±0.00	2.6±0.00	8.64±0.00
2368/1	7.3±0.00	8.4±0.00	5.7±0.00	12.5±0.00	0.148±0.00	2.2±0.00	12.96±0.00
2368/2	7.4±0.00	8.5±0.00	4.4±0.00	16.9±0.00	0.153±0.00	2.1±0.00	14.96±0.00
Range	6.7-7.4	7.6-8.5	4.42-16.33	12.46-16.95	0.148-0.200	2.1-2.9	4.32-14.96

The results of the tested Fluvisol soils showed that they are characterized by the following features: neutral to alkaline reaction (pH in 1M KCl), slightly to medium alkaline reaction (pH in H_2O), medium to highly carbonated, very low to medium provided with available P, low to medium provided with available K, and medium provided with total N and humus (Džamić et al., 1996).

According to the share of clay, sand, and silt fractions in both soils, 94% of the examined samples belongs to the textural class of light clays, 2% belongs to heavy clay, while 1% each belongs to clay loam, silty loam, silty clay loam and silty clay.

Based on the terrain reconnaissance and the results obtained in this research from the depth of 30 cm, it was established that the CM Progar agricultural areas are suitable for intensive agricultural production, namely the cultivation of wheat, maize and sunflowers. Therefore, general recommendations for fertilisers and liming materials application, including the type, dosage and time of application, pH value, the state of calcium in the soil, as well as other soil fertility parameters (Popović, 1989), are given for the mentioned field plants.

The general recommendation for the lime material introduction, in the amount of 4-6 t $CaCO_3$ ha^{-1} (in basic tillage) refers to the following parcels: 935/1, 941, 947/1, 952, 953, 962, 943, 947/11, 939/33, 939/39, 956, 942, 945, 960, 976, 946, 970.

The general recommendations for mineral fertilisation of wheat (in the fall during the basic tillage; the fertilizers amounts for plant nutrition depend on the available N in February-March) are as follows: 500 kg ha^{-1} NPK 16:16:16 (parcels: 935/1, 935/41, 941, 947/1, 952, 953, 962, 2334/2); 300 kg ha^{-1} NPK 10:30:20 (parcels: 939/1, 943, 947/11); 300 kg ha^{-1} NPK 10:30:20 + 100 kg ha^{-1} MAP (parcels: 939/33, 939/39, 956); 600 kg ha^{-1} NPK 10:30:20 (parcel 942); 400 kg ha^{-1} NPK 16:16:16 + 100 kg ha^{-1} MAP (parcels: 945, 960, 976, 2335/1, 2335/2, 2368/1, 2368/2); 500 kg ha^{-1} NPK 16:16:16 + 100 kg ha^{-1} MAP (parcels: 946, 970).

The general recommendations for mineral fertilisation of maize (before sowing) are as follows: 400 kg ha^{-1} NPK 10:30:20 + 300 kg ha^{-1} KAN (parcels: 935/1, 935/41, 939/33, 941, 945, 947/1, 952, 953, 956, 960, 962, 976, 2334/2, 2335/1, 2335/2, 2368/1, 2368/2); 500 kg ha^{-1} NPK 16:16:16 + 200 kg ha^{-1} KAN (parcels: 939/1, 943, 947/11); 350 kg ha^{-1} NPK 10:30:20 + 250 kg ha^{-1} KAN (parcel: 939/39); 600 kg ha^{-1} NPK 16:16:16 + 100 kg ha^{-1} MAP (parcel: 942); 500 kg ha^{-1} NPK 10:30:20 + 200 kg ha^{-1} KAN (parcel: 946); 500 kg ha^{-1} NPK 10:30:20 (parcel: 970).

The general recommendations for mineral fertilisation of sunflower (before sowing) are as follows: 600 kg ha^{-1} NPK 16:16:16 + 100 kg ha^{-1} KAN + 100 kg ha^{-1} of potassium salt (parcels: 935/1, 935/41, 941, 947/1, 952, 953, 962, 2334/2); 400 kg ha^{-1} NPK 6:12:24 + 200 kg ha^{-1} KAN + 100 kg ha^{-1} MAP (parcels: 939/1, 943, 947/11); 600 kg ha^{-1} NPK 16:16:16 + 100 kg ha^{-1} KAN (parcels: 939/33, 945, 956, 960, 976, 2335/1, 2335/2, 2368/1, 2368/2); 500 kg ha^{-1} NPK 16:16:16 + 150 kg ha^{-1} KAN (parcel: 939/39); 800 kg ha^{-1} NPK 16:16:16 (parcel: 942); 700 kg ha^{-1} NPK 16:16:16 + 100 kg ha^{-1} MAP (parcels: 946, 970).

Conclusion

The results of soil chemical parameters and textural class indicate that the examined soils of Progar, Surčin Municipality, up to depth of 30 cm, are mostly suitable for intensive field production, mainly wheat, maize and sunflower. Accordingly, general recommendations for fertilisers and liming materials application, such as type, dosage and time of application, pH value, the state of Ca in the soil, and other soil fertility parameters, are given for the mentioned plants. It is necessary to regularly monitor the changes in the basic chemical parameters of the tested soils fertility in order to give appropriate recommendations based on the obtained results.

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GRAIN YIELD AND YIELD COMPONENTS OF PROSPECTIVE HOMOZYGOUS WINTER WHEAT GENOTYPES

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Abstract: During the two-year research on the experimental field of the Grain Center in Kragujevac, the 1,000-grain weight, hectoliter mass and grain yield of ten prospective homozygous genotypes of wheat were analyzed. During the growing season, the average annual temperature and precipitation were favorable for growing wheat. In both growing seasons, precipitation was 120 mm more than the annual average, and 220 mm more in the second year. Genotype L-1/59 had the highest grain yield and the highest 1,000-grain weight, while the hectoliter mass was more or less equal to other genotypes. All genotypes had a satisfactory 1,000-grain weight ranging from 43 to 50 g. On average, all analyzed lines had a hectoliter mass greater than 80 for both years, which ranks them as high quality.

Keywords: grain yield, 1,000-grain weight, hectoliter mass, wheat

Introduction

The main task of breeding is the creation of hybrids and varieties of plants that, with optimal cultivation technology in specific environmental conditions, would have a high potential for yield. In the Republic of Serbia, in the last 20 years, grain yields have varied significantly, ranging from very low 2.2 t ha^{-1} in 2003. to 5.7 t ha^{-1} in 2021. The large fluctuations in yields indicate that we still depend to a large extent on climatic conditions, meaning that in climatically favorable years, we achieve relatively good yields, while in unfavorable years, yields are very poor (Dodig et al., 2015; Đurić et al., 2020). Factors that will be the main obstacles in the fight for higher production are unfortunately becoming more prevalent worldwide (Knežević et al., 2020). The reduction of freshwater quantity and its increasing contamination with various toxic and destructive substances will result in a further decrease in the already small

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areas under wheat irrigation. Soil degradation, both in terms of chemistry (increased acidity and salinity) and functionality, is present on large areas worldwide (Denčić et al., 2009). The emergence of new diseases and pests (such as the new race of stem rust Ug99) that can reduce yields by up to 50% is also a significant challenge (Braun et al., 2008). One of the extremely negative consequences of global warming will be heat waves, which in the grain filling phase can have catastrophic negative effects on both yield and quality. The higher cost of energy will undoubtedly lead to an increase in the prices of all inputs and, logically, producers will either significantly reduce the use of mineral fertilizers or refrain from their use altogether (Sayre and Hobbs, 2004). In the Republic of Serbia, compared to the ten-year average (2011–2020), cultivated areas of wheat increased by 9.4%, and average wheat yields range from 4.5 to 8 t ha^{-1}. Numerous traits play a key role in determining yield, and the contribution of each trait may vary among different genotypes and environmental conditions due to interactions within each genotype and between genotypes and environmental conditions (Đekić et al., 2015; Zuo et al., 2017; Grčak et al., 2019).

The work aimed to analyze the variability of the yield components, as well as the grain yield of prospective homozygous wheat lines.

Materials and methods

In the experimental field of the Center for Small Grains and Rural Development in Kragujevac, during a two-year study (2018/19 and 2019/20), grain yield, hectoliter mass and weight of 1,000 grains were determined for ten homozygous wheat lines: L-2/6, L-5/98, L-5/7, L-6/7, L-4/4, L-2/24, L-2/2, L-1/5 - 9, L-19/54 and L-3-2/3. The trial was conducted in a 5 m^2 plot with 10 rows, 12.5 cm between rows and 3 cm between plants in a row, according to a randomized block design with four replications. In both years, maize was used as a previous crop. The trial was sown in the second half of October (optimal time). Before sowing, 400 kg ha^{-1} of NPK (8:16:24) fertilizer was applied, and in the spring fertilization, an additional 300 kg ha^{-1} of ammonium nitrate (KAN) was added.

Meteorological conditions

The average annual air temperature in the first year of the research was 0.49°C higher than the thirty-year average, and in the second year it was higher by almost 1°C. Amount of precipitation for Kragujevac during the 2019/20 growing season it was 728.5 mm, which is 220 mm more than the multi-year average. The most precipitation in 2019 and 2020 was in June, with 143 mm in

2019 and 192.9 mm in 2020. Both the average annual temperature and the amount of precipitation in both growing seasons were above the long-term average (Table 1).

Table 1. Average monthly temperature and precipitation amount

	Average monthly temperature(°C)			*Precipitation amount* (mm)		
Month	2018/19	2019/20	1981- 2010	2018/19	2019/20	1981-2010
X	13.9	13.6	11.9	9.4	196	48.9
XI	7.6	11.7	6.4	41.8	68.1	49.5
XII	2.6	4.9	2.1	51.8	57.6	45.8
I	0.1	1.3	0.9	85.3	23.3	37.9
II	4.2	6.2	2.3	2.2	47.6	37.0
III	9.1	7.8	6.6	10	55.7	42.3
IV	13.2	11.8	11.7	35.2	17.8	53.9
V	14.5	15.7	16.7	125.3	7.9	58.7
VI	22.4	19.9	20.0	143	192.9	76.4
VII	22.3	22.0	21.9	83.2	61.6	57.7
Average/Sum	10.97	11.49	10.5	627.2	728.5	508.1

Results and discussion

Grain yield is a complex trait of exceptional economic importance, depending on several inherited characteristics and environmental conditions in which plants develop. Therefore, numerous researches in the field of genetics and plant breeding aim to contribute to a better understanding of both yield and its components, especially crops grown for grain production. Wheat yield and its components are quantitative traits that are significantly influenced by environmental factors and applied agronomic practices during the growth cycle of the wheat plant (Jaisi et al., 2021; Urošević et al., 2023).

The prospective wheat genotypes exhibited variations in grain yield, showing inconsistency across the years (Table 2). When observed year by year, there were no significant differences in grain yield, but the lines significantly differed from each other in terms of yield. Line L-1/59 had the highest average yield over the two years, followed by L-2/2, while the lowest yield was observed in line L-63/7. In the last 20 years in Serbia, wheat grain yields have varied significantly, ranging from 2.2 t ha^{-1} to 4.5 t ha^{-1} (Denčić et al., 2009). This yield variation indicates that grain yield is highly dependent on climatic conditions. Growing conditions (year) significantly influenced yield, and a significant interaction effect between genotype and year was observed (Zečević et al., 2010; Knežević et al., 2022; Matković Stojšin et al., 2022).

Hectoliter mass represents the weight of one hectoliter of wheat expressed in kilograms and is accepted as a measure of wheat quality due to its simplicity and quick determination. It can also serve as an indicative value for evaluating milling quality and ranges from 60 to 84 kg in wheat, with good wheat needing to have a hectoliter weight above 76 kg. All analyzed lines, on average for both years, had a hectoliter weight greater than 80, classifying them as lines of good quality (Table 2). Comparing the results of yield and hectoliter weight in this study, it is evident that the varieties that achieved high yields also had higher hectoliter mass.

Table 2. Grain yield, hectolitre mass and 1,000-grain weight of wheat genotypes

Genotype (A)	Grain yield (kg ha^{-1})			Hectolitre mass (kg hL^{-1})			1,000-grain weight (g)		
	Years (B)			Years (B)			Years (B)		
	18/19	19/20	*Average*	18/19	19/20	*Average*	18/19	19/20	*Average*
L-2/6	6361	5808	6084	81.1	79.5	80.3	44.0	42.36	43.18
L-5/98	6532	6200	6365	82.1	82.9	82.5	44.5	47.74	46.12
L-5/7	6000	6462	6231	82.1	83.3	82.7	44.35	44.52	44.44
L-6/7	5715	5978	5846	80.3	82.3	81.3	44.10	44.84	44.47
L-4/4	6090	5960	6025	80.3	83.3	81.8	45.70	46.86	46.28
L-2/24	6580	5833	6206	81.7	82.5	82.1	45.25	50.52	47.88
L-2/2	6710	6120	6415	85.5	82.3	83.9	47.30	44.56	45.93
L-1/59	6320	6650	6485	83.3	81.1	82.2	50.38	50.82	50.6
L-1/54	5870	6540	6205	84.9	83.3	84.1	47.48	47.26	47.37
L-32/3	5705	6420	6062	85.3	83.5	84.4	42.53	45.80	44.16
Average	6188.3	6197.1		82.66	82.4		45.56	46.51	
	A	B	AxB	A	B	AxB	A	B	AxB
LSD_{005}	196.7	107.4	274.3	0.25	0.17	0.46	0.27	0.19	0.50
LSD_{001}	340.9	182.5	457.3	0.42	0.29	0.79	0.46	0.32	0.86

The 1,000-grain weight depends on the variety, agroecological conditions, and production technology. Line L-1/59 had the highest 1,000-grain weight (over 50g) in both years, while the lowest was observed in line L-2/6. A weight of 1000 grains of 50g is an indicator of high grain yield (Kovačević and Rastija, 2014). Wheat variety is one of the most significant quality factors influenced by variations in the harvest year, region, and location (Kaya et al., 2014; Đekić et al., 2015). Growing conditions (year) significantly influenced yield, and a significant interaction effect between genotype and year was also observed.

Conclusion

Weather conditions in both observed growing seasons were relatively favourable for growing wheat, with average annual temperature and precipitation above multi-year averages. During the winter months in the observed period, there was significantly less precipitation, and the winter was mild, which positively affected wheat development and enabled high yields. In terms of yield, the best results were achieved by the L-1/59 line. This variety also had the highest 1,000-grains weight, while the hectoliter mass was comparable to other genotypes. All genotypes had a satisfactory 1,000-grains weight, ranging from 43 to 50 g. All potential future varieties had an average hectoliter weight of more than 80 kg for both years, which ranks them as good-quality lines.

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EFFECTS OF LIME ON ALFALFA CULTIVARS YIELD ON ACIDIC SOIL

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Abstract: Soil acidity limits the cultivation of alfalfa and the effecting of high yields. The experiment was set up on acidic soil to determine the effect of lime (0, 1 t ha^{-1}, 2.5 t ha^{-1}) on forage yield of alfalfa cultivars (K-28, Zuzana, NS-Nijagara) in the year of establishment. The yield of the liming variants was higher in all three cut and total yield, compared to the control. The significant differences in yield were only in the third cut, where the K-28 (4.76 t ha^{-1}) and NS-Nijagara (4.46 t ha^{-1}) had a higher yield compared to the Zuzana (3.65 t ha^{-1}). Interaction cultivar/lime was significant only in the first cut, while in the other two cuts and in the total yield it wasn't.

Keywords: acidic soil, alfalfa, cultivars, lime, yield

Introduction

Alfalfa (*Medicago sativa* L.) is one of the most important forage crops, cultivated on over 33 million hectares worldwide, due to its high nutritional value and high yield (Radović et al., 2009). In the Republic of Serbia, the total growing area in 2021 was 106.340 ha, with an average hay yield of 5.1 t ha^{-1} (Statistical yearbook of the Republic of Serbia, 2022). The alfalfa dry matter yield in the Republic of Serbia in drought conditions varies between 24 - 30 t ha^{-1} (Milić et al., 2014). Alfalfa has high soil requirements (Jakšić, 2014), and the main limiting factors for cultivation are the soil acidity and the toxicity of the Al^{3+} ions (Khu et al., 2012). Soil acidity is a major issue in the Republic of Serbia, around 60% of the arable land has moderately to extremely acidic soil, with a pH range of 4.5 to 6.0 (Dugalić et al., 2012). It has a negative impact on the emergence of seedlings, their early growth, and overall biomass production (Stevović et al., 2010). One of the ways to increase the area to growing alfalfa in our production conditions can be effetced by calcification (Stevović et al., 2012),

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with regular application of organic and mineral fertilizers before sowing (Stojiljković et al., 2021). The most commonly used materials are ground limestone (50-55% CaO), dolomite (30% CaO), calcium oxide (70-90% CaO), hydrated lime (60-70% CaO), saturation mud (22% CaO), and other industrial by-products (Katić et al., 2007).

Different cultivars react differently to soil acidity and choice of tolerant cultivars should be considered as a factor in overcoming acidity issues (Stevović et al., 2012). According to Grewal and Williams (2003), there is a variation in alfalfa cultivars reaction to high soil acidity.

This research was aimed to evaluate the effect of soil calcification on forage yield in three cultivars of alfalfa in the year of establishment.

Materials and methods

The experiment was conducted in the locality of Mačkovac, (municipality Kruševac 43°33′563″ N, 21°13′608″ E; 247 m). The soil is eutric cambisol, very acidic chemical reaction pH_{KCl} 4.55, (exchangeable acidity is 2.28 m.e/100g of soil), the content of mobile aluminum is 9.45 mg/100g of soil. Soil had high conten of nitrogen 0.25%, moderately provided by phosphorus 11.06 mg/100g, potassium 13.28 mg/100g, humus 2.83% and low carbonate content 1.4% CaO. The experiment was set up as a split plot design with four replications. Hydrated lime (70% CaO) was utilized in amounts of 0 (control), 1 and 2.5 t ha^{-1} for calcification, five months before the sowing. This matterial is spread across the surface and plowed to a depth of 20 cm. Used three cultivars: K-28, Zuzana and NS-Nijagara. Sowing rate was 20 kg ha^{-1}. In the year of establishment three cuts were made in the flowering phase, and the forage yield was measured. The data were processed by two-factor analysis of variance (ANOVA)(Statistica 12 StatSoft), and the least significant differences were determined by the LSD test.

Results and discussion

In the year of establishment, in the first two cuts, the cultivars did not show significant differences in yield, while in the third cut, the K-28 and NS-Nijagara cultivars effected a significantly higher yield compared to the Zuzana (Tabel 1.). There was no significant difference in yield between the cultivars K-28 and NS-Nijagara. Stevović et al. (2010a) have also reported that the K-28 cultivar had a significantly higher yield than the other cultivars in the first year of cultivation.

In the total forage yield (Tabel 1.), the K-28 cultivar had significant higher forage yield compared to the Zuzana, but not than NS-Nijagara. The differences in the total yield between Zuzana and NS-Nijagara are not significant.

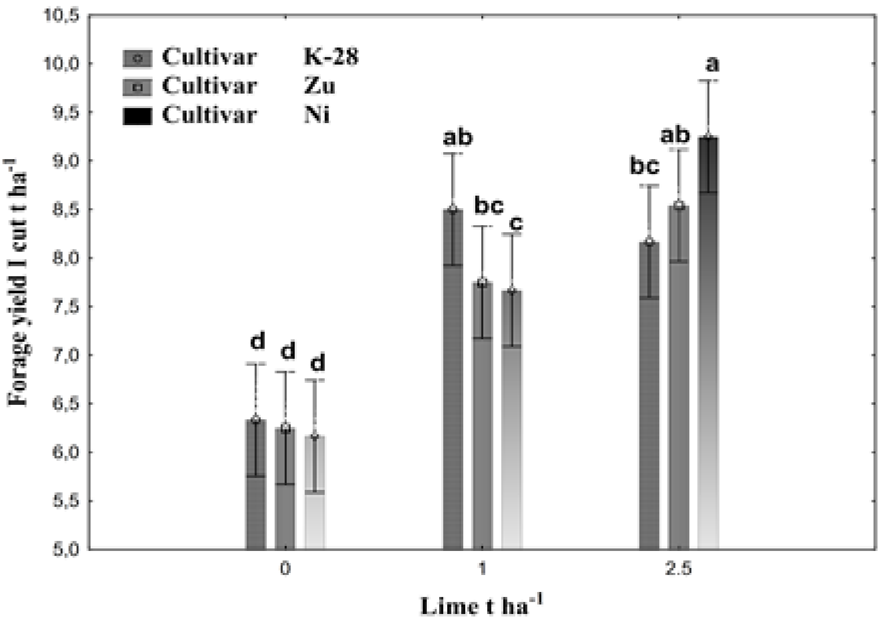
Table 1. Forage yield in the first year (t ha^{-1})

	I cut	II cut	III cut	Total yield
CULTIVAR				
NS-Nijagara	7.69a	12.32a	4.46a	24.47ab
Zuzana	7.51a	12.24a	3.65b	23.40b
K-28	7.67a	12.64a	4.76a	25.07a
LIME				
Control	6.25 c	11.08b	3.76b	21.10b
1 t ha^{-1}	7.97 b	12.96a	4.53a	25.19a
2.5 t ha^{-1}	8.65 a	13.42a	4.58a	26.65a
ANOVA				
Cultivar	**ns**	**ns**	*	*
Lime	*	*	*	*
Cultivar x lime	*	**ns**	**ns**	**ns**

Values in columns with a different letter are significantly different based on the LSD test (R<0.05)
*F-test significant at R≤0.05; ns-not significant

The application of 1 t ha^{-1} and 2.5 t ha^{-1} CaO increased the alfalfa yield in all three cuts and the total yield compared to the control (without lime) in the year of establishment of alfalfa. In the first cut on the variant with a higher dose of lime, 2.5 t ha^{-1}, a significantly higher yield was effected compared to 1 t ha^{-1} (Tabel 1.). Similar results were reported by other authors, where calcification also affected significantly higher alfalfa biomass yields in the year of establishment compared to the control (Dugalić et al., 2012; Stevović et al., 2004, 2010, 2010a). Katić et al. (2006) also reported a significant increase in yield on pseudogley soil, active acidity 4.79, with the application of 3 t ha^{-1} and 6 t ha^{-1} powdered lime (70% CaO) in the first year. However, Popović et al. (2007) did not see significant variations in yield in the first year, using different doses of dolomite (56% CaO) on soil pH$_{KCl}$ 4.60. The authors reported that the weak effects of lime in the first year are attributed to the lime material (dolomite) which is less soluble than powdered lime. Also, Hendry et al. (2018) in their research did not find significant differences in alfalfa yield in the first year of the trial.

Cultivars reacted differently to the introduction of lime into the soil. The cultivar/lime interaction showed that a significantly higher alfalfa forage yield was observed only in the first cut (Graph 1). In the control treatment, the differences between the cultivars were not significant. With the addition of 1 t ha^{-1} lime K-28 had a higher forage yield than NS-Nijagara, but the differences in yield between Zuzana and NS-Nijagara and between K-28 and Zuzana are not significant. Applying 2.5 t ha-1 of lime significantly increased the forage yield of the NS-Nijagara variety compared to K-28, while there was not difference in yield between Zuzana and NS-Nijagara, nor between K-28 and Zuzana. In the other two cuts, significant.



Graph 1. Forage yield cultiltivars/lime in the first cut (t ha^{-1})

Stević et al. (2010a) have calculated the degree of tolerance to acidity of alfalfa cultivars Banat, NS-Medijana, K-28, Sinaskaja and OS-66. The authors reported that in the year of establishment of the control treatments, there was no difference between the cultivars, and that increasing the amount of CaO to 1.5 t ha^{-1} and 3 t ha^{-1} led to an increase in yield in all cultivars. The best reaction was observed in the cultivar K-28, while the other cultivars showed a weaker reaction to the application of an increased amount of CaO.

Conclusion

The application of different doses of lime led to an increase in the alfalfa yield in all three cuts and the total forage yield.

In the third cut, the cultivars K-28 and NS-Nijagara achieved a significantly higher yield compared to Zuzana. Also, in the total yield, the cultivar K-28 had a significantly higher yield than Zuzana, but not than NS-Nijagara.

The cultivar/lime interaction shows that the cultivars reacted to differents amounts of lime only in the first cut.

For growing alfalfa in acidic soil, it is recommended to increase the amount of lime to 2.5 t ha^{-1} and select cultivas such as K-28 and NS-Niagara. More precise research results will be confirmed in the coming years.

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THE EFFECT OF CLIMATE FACTORS ON MAIZE YIELD OF LATE SOWING DATE

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Abstract: Two-year research was carried out in 2014 and 2015 on the territory of the municipality of Leskovac, on alluvium land type. In the experiment, 6 maize hybrids of different vegetation lengths 400-600 were sown (ZP 434, NS 4023, ZP 555, NS 5051, ZP 666, NS 6030). The hybrid NS 6030 (10.248 t ha^{-1}) had the highest average yield in 2014, while the hybrid FAO group 400 NS 4023 (9.467 t ha^{-1}) had the lowest. The highest average yield in the year 2015, unfavorable for corn production, was achieved by the hybrid FAO group 400 ZP 434 (6.938 t ha^{-1}), and the smallest yield by hybrid FAO group 600 NS 6030 (4.980 t ha-1). In 2015, being that the average temperatures were warmer than in 2014, and it had fewer amounts and worse distribution of precipitation, late sowing had a very bad effect on all hybrids, especially on FAO 600 hybrids.

Keywords: corn, precipitation, temperatures, sowing time, yield

Introduction

Meteorological conditions have the greatest influence on the stability of corn yields, primarily the amount and distribution of precipitation during the vegetation period of plants, as shown by the results of numerous studies (Branković-Radojčić et al., 2017; Ikanović et al., 2018; Božović et al., 2020; Madić et al., 2021).

Sowing time is one of the factors that have a significant impact on the level of corn yield (Glamočlija and Ugrenović, 2016; Biberdzić et al., 2018; Stojiljković, 2022). The optimal sowing period which could be defined as the sowing time, ensures the sprouting of corn at the most favorable moment, to make use of the conditions of the growing season and form the highest possible yield (Videnović et al., 2011). To achieve high and stable yields, numerous

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researchers have studied the vegetation length of certain corn hybrids in different weather conditions from germination to maturation, because not all hybrids can be grown equally successfully in all production areas (Babić et al. 2013; Grčak et al., 2020; Petrović et al., 2023).

It very often happens that producers are late with corn sowing due to organizational reasons or weather conditions, so, it was necessary, based on the results of the experiment, to guide them on which hybrids are recommended to choose in conditions of late sowing.

The goal of the research was to determine the effects of hybrids of different FAO ripening groups and climatic conditions on the yield of certain corn hybrids in the late sowing period.

Materials and methods

The research was carried out in 2014 and 2015 in the area of the municipality of Leskovac. 6 maize hybrids of FAO group 400-600 were included in the experiment, namely: FAO 400 (ZP 434, NS 4023); FAO 500 (ZP 555, NS 5051) and FAO 600 (ZP 666, NS 6030).

Sowing of corn hybrids was done on April 30 according to a random block system, in three repetitions. 4 rows of each hybrid were sown with an inner-row distance of 70 cm. FAO group 400 hybrids were sown with a distance between plants of 20 cm, FAO 500 (25 cm), and FAO 600 (30 cm).

The basic processing and fertilization were carried out based on the agrochemical analysis of the soil in the autumn-winter period. Pre-sowing preparation was done with a disc harrow in two passes on the day of sowing. The recommended amount of nitrogen fertilizer for feeding (200 kg ha^{-1} KAN) is divided into two feedings. The first feeding was done when the plants were in the stage of 3-4 leaves, with 60% of the recommended amount for feeding. The second feeding was done with the remaining amount of KAN when the corn was in the stage of 7-8 formed leaves. When the crop was in the stage of 6 formed leaves, the crop was protected against weeds with herbicides (Motivell, in the amount of 0.75 l ha^{-1} + Callisto 0.25 l ha^{-1}).

The yield was calculated and reduced to 14% moisture and statistically processed using the IBM SPSS program version 21.

Results and discussion

The Leskovac meteorological station is located near the test plots and served us to obtain data on average daily temperatures and precipitation (table 1).

Table 1. Average monthly air temperatures (°C) and precipitation (mm) in Leskovac (2014-2015)

	April	May	June	July	Aug.	Sept.	A./S.
Average monthly temperatures and precipitation during 2014							
mm	214	117	64.3	86	47.1	121.2	464.1
°C	11.40	15.55	19.59	21.60	21.50	17.00	17.77
Average monthly temperatures and precipitation during 2015							
mm	64.5	38	33	6	63.2	51.2	255.9
°C	10.78	18.03	19.60	24	23.50	20.00	19.31
Average monthly temperatures and precipitation - ten-year average							
mm	50	60.8	61.2	38.7	32.7	64.8	308.2
°C	12.30	16.20	20.3	22.8	22.2	17.4	18.53

Based on the data from Table 1, we can notice that during the two-year research and the ten-year average of temperature and precipitation, differences were observed both in terms of the total amount of precipitation during the growing season, and in terms of the distribution of precipitation by month. The total amount of precipitation during the growing season in 2014 was 464.1 mm, with an average temperature of 17.77 °C. It is particularly important to note that 197 mm of precipitation fell in June, July, and August, which is 65 mm more than the multi-year average for the same period. This period is of particular importance for the process of formation of corn yield. Based on the data on the amount of precipitation and average temperatures, we can state that this year was favorable for corn production. This is supported by the fact that 155.9 mm more precipitation fell in 2014 than the ten-year average.

In 2015, during the growing season, 255.9 mm fell, which is 208.2 mm less than in 2014, while the average monthly temperature during the growing season was 19.31°C, which is 1.54°C higher than in 2014. During 2015, it fell 52.3 mm less compared to the ten-year average, and the average temperature in 2015 was 0.78°C higher than the ten-year average. A particularly unfavorable period was during June, July, and August, with 102.8 mm of precipitation, and an average monthly temperature of 22.36°C during these three months, which classifies the year 2015 as extremely unfavorable for corn production. The importance of production conditions, especially temperature, and precipitation, is crucial according to Maitah et al. (2021), who claim that yield is negatively correlated with temperatures during July and August.

Table 2. Yield of corn hybrids of different FAO ripening groups (t ha^{-1}) in 2014 and 2015

Yield of corn hybrids of different FAO ripening groups (t ha^{-1}) in 2014						
	H1	H2	H3	H4	H5	H6
Mean	9.717	9.467	9.989	10.057	10.142	10.249
Median	9.692	9.471	9.967	10.053	10.208	10.288
Mode	9.58[a]	9.36[a]	9.89[a]	9.96[a]	9.90[a]	10.15[a]
Std. Deviation	0.152	0.103	0.107	0.098	0.220	0.083
Variance	0.023	0.011	0.011	0.010	0.048	0.007
Minimum	9.58	9.36	9.89	9.96	9.90	10,15
Maximum	9.88	9.57	10.11	10.16	10.32	10.31
Yield of corn hybrids of different FAO ripening groups (t ha^{-1}) in 2015						
Mean	6.938	6.912	6.135	5.725	5.307	4.980
Median	6.954	7.005	6.156	5.750	5.450	4.920
Mode	6.75[a]	6.45[a]	5.35[a]	5.42[a]	4.88[a]	4.67[a]
Std. Deviation	0.180	0.422	0.775	0.293	0.376	0.344
Variance	0.033	0.178	0.601	0.086	0.141	0.118
Minimum	6.75	6.45	5.35	5.42	4.88	4.67
Maximum	7.11	7.28	6.90	6.01	5.59	5.35

The average yield for all hybrids in 2014, which according to the amount of precipitation, especially in June, July, and August, was above the long-term average, was 9.936 t ha^{-1}. The highest average yield was achieved by hybrid NS 6030 (10.249 t ha^{-1}) and the lowest hybrid FAO group 400 NS 4023 (9.467 t ha^{-1}). The average yield in 2015 was 5.999 t ha^{-1}. The highest average yield was achieved by the hybrid of FAO group 400 ZP 434 (6.938 t ha^{-1}), and the lowest hybrid of FAO group 600 NS 6030 (4.980 t ha^{-1}). The year 2015 had average temperatures warmer than the year 2014 and also had a smaller amount and worse distribution of precipitation, so late sowing had a very bad effect on all hybrids by reducing yields, especially on hybrids FAO 600. Based on the results of numerous research and stability analyses of maize yields in Republic of Serbia, hybrids of mid-early ripening groups show better adaptability to unfavorable conditions during the growing season, while hybrids of later ripening groups achieve higher yields and exhibit positive characteristics in more favorable production conditions (Ikanović et al., 2018; Božović et al., 2020) which is in agreement with our experimental results.

Table 3. Comparison of the influence of hybrids and sowing year on grain yield (t ha^{-1}) in 2014 and 2015

H_2014-H_2015	Average values	Std. Deviation	Std. Error	95% Confidence Interval of the Difference		t	p
H1	8.327	0.305	0.176	2.021	3.536	15.780	0.004
H2	8.190	0.321	0.185	1.758	3.352	13.791	0.005
H3	8.062	0.881	0.509	1.664	6.042	7.573	0.017
H4	7.891	0.196	0.113	3.845	4.819	38.259	0.001
H5	7.725	0.532	0.307	3.514	6.156	15.747	0.004
H6	7.615	0.333	0.192	4.442	6.096	27.409	0.001

From a total of 6 pairs used for comparison (all 6 hybrids in both 2014 and 2015), all results had a statistically significant difference (table 3). In all cases, the yields of corn in 2014 were higher than in 2015, which indicates that the weather has an important influence in determining the yield of corn grains. The difference in yield over the years was more pronounced in the hybrids of FAO group 600. Meteorological conditions had a great influence on the yield. Hybrids with a shorter growing season achieved satisfactory yields in 2015, which was unfavorable for corn production. The reason for this is the earlier passing of the stages of silking, pouring, and grain formation. That the weather conditions affect the yield is stated by Filipović et al., 2015, adding that the average annual decrease in yield caused by drought, and other stress factors associated with it, ranges from 10% to 20%, even up to 50% compared to expected yields.

Conclusion

Based on the results of a two-year study on the influence of climatic factors on the yield of corn in the late sowing period, we conclude the following:

The yields of all corn hybrids in 2014 were higher than in 2015, which indicates that the weather has an important impact on determining the yield of corn grains. The average yield for all hybrids in 2014 was 9.936 t ha^{-1}, while in 2015, which was unfavorable in terms of the amount and distribution of precipitation, an average yield of 5.999 t ha^{-1} was achieved.

The hybrid NS 6030 had the highest average yield in 2014, and the hybrid FAO group 400 NS had the lowest. The highest average yield in 2015 was achieved by the hybrid of FAO group 400 ZP 434, and the lowest hybrid of FAO group 600 NS 6030.

Bearing in mind that corn production mostly takes place in dry tillage and late sowing conditions, it is recommended to sow hybrids with a shorter vegetation period in which the stages of threshing, silking, and grain formation occur faster.

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ADVANCING THE NEW RESEARCH PATHWAYS OF "NUTRIBREEDING" FOR HUMAN AND ANIMAL NUTRITION WITH HEALTH BENEFITS

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Abstract: New pathways in grain breeding and the cultivation of nutritionally enriched crops are imposed by the world's population expansion and trends in food and feed production, which encourage the use of functional foods with potential health benefits. A sustainable turnkey system that produces high-quality, nutritionally rich crops may be achieved by a new generation of genotypes of cereals and legumes, such as soybean and maize, that have optimal physical and chemical properties along with higher antioxidant levels due to the presence of polyphenols and anthocyanins. Generating the new research avenue called "nutribreeding" is part of the CREDIT Vibes project funded by the European Commission.

Keywords: grains, breeding, food and feed, nutrients, health benefits

Introduction

Maize (*Zea mays* L.) stands as one of the most important cereal crops for utilization as a raw material for food, feed, energy, and industrial applications. Data from Statista (Shahbandeh, 2024) indicate that over the 2023–2024 timeframe, about 1.235,73 million metric tons of maize were produced worldwide. With 388.01 million metric tons produced globally over the 2023–2024 period, soya bean (*Glycine max*) is the most extensively used legume (Shahbandeh, 2024). Despite being a major food crop, maize has seen a sharp growth in demand over the past twenty years for livestock feed. This was mostly brought on by the swift economic growth in highly populated regions of Asia, the Middle East, and South America, which raised the demand for food products made from chicken and cattle among affluent consumers (Shiferaw *et al*., 2011; Delgado 2003). Interest in functional soy products has been on the rise in recent years. The reason for this is the nutritional composition and properties that can favorably affect cardiovascular diseases and the overall health of consumers. The soybean, in addition to being rich in protein, has a low content of saturated fat and does not contain cholesterol.

According to Cederroth and Nef (2009) and Barać *et al*. (2014), soybeans are made up of macronutrients like proteins, lipids, and carbohydrates, micronutrients like vitamins and minerals, and phytochemicals such as phytosterols, tocopherols, carotenoids, and phenolic compounds. Because soy products offer high-quality proteins, few saturated fats, and no cholesterol, they are becoming an increasingly essential source of protein in the human diet and have functional, nutritional, and even health benefits for those with cardiovascular illnesses and general health (Tang, 2019).

Due in large part to the excellent antioxidant activity of the already existent bioactive chemicals in colored grains, interest in their utilization in the food sector has grown. The color of maize grains varies greatly genetically. There are types of maize that are black, blue, purple, pink, red, orange, green, and brown (Žilić et al., 2023). The color difference between black, brown, and yellow (standard) soybeans is caused by the presence of proanthocyanidins and anthocyanins in the seed coat's epidermal layer (Žilić et al., 2013). In Korea, China, and Japan, black soybeans have been regularly used for millennia as a medicinal plant (Xu and Chang, 2008).

The three guidelines of the concept of CREDIT Vibes are nutrient-rich food and feed, a safer, eco-friendly environment, and a healthy existence on an ecologically sound planet. Creating an inventory of genetic resources available for each crop species and defining a working collection of nutricrops are among the key objectives of the project. As a part of the project, colored maize and soybean genotypes with good nutritional and bioactive properties were analyzed to identify the most promising hybrids/varieties presently popular in conventional production, for further research.

This paper tends to showcase some of the recently obtained results on the chemical composition and bioactive properties of the selected maize and soybean genotypes. These represent a promising starting material for further research on the development of nutritionally rich grains for food and feed production.

Materials and methods

The plant material used in this study encompasses three maize and three soybean genotypes created and grown in the experimental plots of the Maize Research Institute, Zemun Polje, Belgrade, Serbia (44°52′N, 20°19′E, 81m asl). The maize grains and soybeans were ground in a laboratory mill (Perten Mill 120 CE; Perten Instruments, Hägersten, Sweden) and a mill with a cooling chamber (Knifetec 1095 Sample Mill, Foss Tecator, Höganäs, Sweden), respectively. The Kjeldahl (AOAC, 1990) and Ewers (ISO 10520:1997) methods were applied to determine the protein and starch content, respectively. The oil content was analyzed according to the Soxhlet method (AOAC, 2000). The Van Soest detergent method modified by Mertens (1992) was used to determine the share of lignocellulosic fractions. The antioxidant capacity of the maize and soybean flour was measured by the direct or QUENCHER method using the ABTS (Serpen *et al*., 2008). Absorbance was measured at 734 nm (Agilent 8453 spectrophotometer; Agilent Technologies, Inc.), and total antioxidant capacity was expressed in mmol Trolox equivalents per kg of dry mass. Determination of total yellow pigment content was performed according to the reference method AACC (1995) 14-50. All analyses were done in two replicates. The results are shown as averages.

Results and discussion

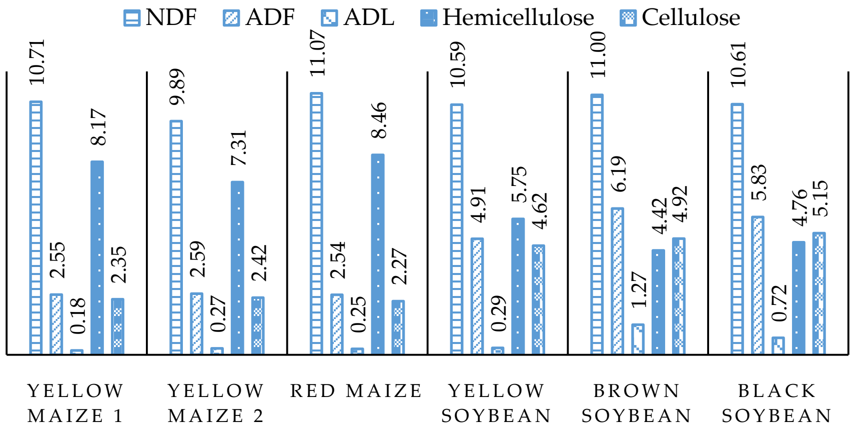
The chemical properties of the investigated maize and soybeans samples are shown in Table 1.

Table 1. Chemical properties of maize and soybean grains (%)

Genotype	Starch	Protein	Oil	Ash
Yellow maize 1	70.32	10.45	5.32	1.56
Yellow maize 2	73.76	10.38	4.85	1.24
Red maize	70.23	11.27	5.27	1.42
Yellow soybean	N. D.	41.15	21.76	5.23
Brown soybean	N. D.	37.45	21.59	5.46
Black soybean	N. D.	42.27	22.44	5.42

The starch content of the maize samples ranged from 70.23% (Red maize) to 73.76% (Yellow maize 2), while the protein content of the soybean genotypes ranged from 37.45% (Brown soybean) to 42.27% (Black soybean).

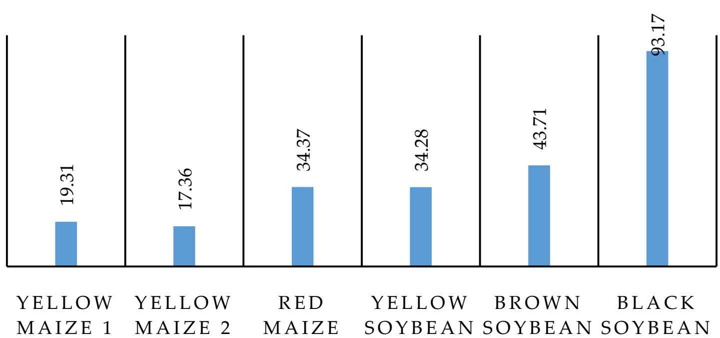
Graph 1 shows the results of analyses of the content of individual lignocellulosic fibers in the tested samples.



Graph 1. Lignocelullosic fibers content (% d.m.)

The genotypes manifested a variation of different lignocellulosic contents, which makes them suitable for different nutritional and industrial applications. (Graph 1). The fiber content in maize and soybeans is one of the important parameters of their nutritional quality. Dietary fiber has become an important ingredient in the modern diet thanks to its beneficial effects on health, such as lowering cholesterol, modifying the glycemic response, lowering insulinemia, improving bowel function, as well as antioxidant properties.

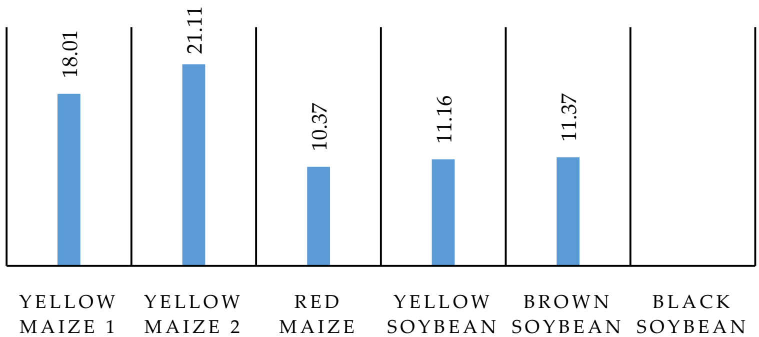
The detected antioxidant capacity of the investigated samples is shown in Graph 2.



Graph 2. Antioxidant capacity (mmol Trolox kg^{-1} d.m.)

The high antioxidant capacity of the black soybean genotype can be attributed to the concentrations of 3-O-glucoside anthocyanins, such as cyanidin, delphinidin, and pelargonidin, make up their seed coat, as previously reported by Žilić et al., 2019. Recent research has demonstrated that dietary fiber and polyphenols from the soybean seed coat can be utilized as bioactive components in pharmaceutical and functional food products targeted at various health issues (Žilić et al., 2020).

The total yellow pigment of the investigated samples was the highest in yellow maize genotype number 2 (21.11 μg βCE g-1 d.m.), while in the black soybean genotype, it was not detected (Graph 3).



Graph 3. Total yellow pigment (μg βCE g^{-1} d.m.)

Conclusion

The maize and soybean genotypes investigated in this study have shown favorable chemical composition and antioxidant properties suitable for different purposes such as the production of nutritious food and feed. They will be used for further research.

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THE INFLUENCE OF EFFECTIVE MICROORGANISMS ON SOME CHARACTERISTICS OF DIFFERENT MAIZE

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Abstract: The aim of the research was to determine the impact of the EM Aktiv preparation in the maize crop (ZP 427 and ZP 548) at 160, 120, 102 kg ha^{-1} N in 2017 at the location of the municipality of Vladimirovci. During the growing season, there was a pronounced water deficit. The preparation was applied in three variants: EM1 - control, EM2 - foliar 2 x 6 l ha^{-1} in the phenological stages of 5-7 leaves and after 15 days; EM3 - the preparation was introduced into the soil 7 days before sowing 30 l ha^{-1} + EM2. The mass of 1000 grains, the height of the yield and the nitrogen content in the grain were determined. Application of 160 kg ha^{-1} nitrogen had the greatest effect on the weight of 1000 grains, and 120 kg ha^{-1} N on grain yield in all treatments. The treatments had a positive effect on both traits. Higher values of the tested properties were found in the EM3 treatment. The applied factors did not have a statistically significant effect on the nitrogen content of maize grains.

Keywords: hybrids, fertilization, biostimulator, yield components

Introduction

Maize represents a strategically very important food plant species, and the demand for maize is growing. Predictions show that the need for this culture will double by 2050 in developing countries (Rosegrant et al., 2008).

The production of maize on a global level takes place in a way that affects the degradation of resources and elements of the environment, the increasing dependence on mineral fertilizers and pesticides, which leads to the production of greenhouse gases and a negative impact on climate change. Today,

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agricultural production emphasizes the management of ecological and biological processes in order to obtain acceptable crop yields and environmental protection. In recent years, there has been an increasing number of studies on the impact of different biostimulants within the fertilization program as a supplement to fertilization (Zamudio et al., 2018). Tejada et al. (2018) investigated the application of different types of foliar biostimulants in the maize crop and obtained positive results of agronomic characteristics and maize yield. Positive results were obtained by Cvijanović et al., (2007) in the examination of the application of diazotrophs on the yield of maize grains and the composition of the rhizosphere microflora. The aim of the work was to examine the influence of a microbiological preparation with effective microorganisms on the mass of 1000 grains, the height of the yield and the nitrogen content in the grains of different genotypes of maize in the dry year of 2017.

Materials and methods

Experimental research was conducted in 2017 on a private plot in the municipality of Vladimirci. The area of the elementary plot was 14 m^2. The plots were laid out according to the plan of divided plots in three repetitions. All agrotechnical measures were applied in optimal terms. (Factor A): to ensure the necessary amount of nitrogen on the entire plot, 30 t ha^{-1} of manure and 300 kg ha^{-1} of complete mineral NPK fertilizer of the formulation 15:15:15 were plowed in autumn. In the course of pre-sowing preparation and one top dressing, which was carried out in the phenological phase of plant development of 5-7 leaves, the following amounts of nitrogen were provided 160 kg ha^{-1}; 120 kg ha^{-1}; 102 kg ha^{-1}.

Two hybrids (factor B) of the toothed yellow grain type were sown, the selection of the Maize Research Institute Zemun Polje ZP 427 and ZP 548. Various treatments were applied with the preparation EM Aktiv (trade name) (Factor C). Treatments: EM1 - no treatment, EM2 - EM Aktiv was used during the growing season 2 x 6 l ha^{-1} in the phenological stages of 5-7 leaves and after 15 days; EM3 - EM Aktiv was introduced into the soil 7 days before sowing, 30 l ha^{-1} + EM2.

EM Aktiv is a liquid preparation containing a mixture of highly effective strains of lactic acid fermentation bacteria, sulfate-reducing bacteria, fungi, yeasts and actinomycetes. These microbes produce a large amount of biostimulatory compounds such as hormones, indole-3 acetic acid, organic acids, antibiotics and B vitamins.

The water regime during the vegetation period was very unfavorable (360.3 mm). 55.2 mm of rain was recorded in April, and 90.2 mm in May, which was more than the conditional-optimal needs for the initial stages of maize growth. In the month of June, only 14.8 mm of rain fell, which is almost six times less than the conditionally optimal needs (90 mm). In July, 49.3 mm of water precipitation fell, which was insufficient, and the dry period continued in August, as 25.5 mm fell, which is four times less than the conditional-optimal needs. In September, 83.7 mm fell, which was 3.74 mm more than needed.The average air temperature in the growing season was 18.3°C. The month of April had an average temperature of 11.5°C, which is lower than optimal needs. In May, the average temperatures were at the level of the required temperatures, while in June the average daily temperatures of 22.7°C were 2.7°C higher, which with a small amount of precipitation of only 14.8 mm was an unfavorable period for the development of generative organs in maize. In August, the average mean daily temperatures were at the optimal level of 23°C, but the period of water deficit continued, so it can be said that the conditions for maize fertilization were unfavorable.

Total proteins were determined by the method micro–Kjeldalh Laboratory for soil and agroecology at the Institute of Crop and Vegetable Farming Novi Sad. To evaluate data we used descriptive statistics and analysis of variance (ANOVA) u programu DSAASTAT Three-way ANOVA was used to test effects of mineral nitrogenin fertylizer, genotype, treatment and growing season. All results were calculated at a significance level LSD of 0.01 and 0.05.

Results and discussion

Based on the processed results, the average weight of 1000 maize grains was determined to be 222.75 g (Table 1).

Table 1. Mass of 1000 grains of maize (g)

N kg ha^{-1} (A)	Genotypes (B)	Treatments (C)			$\bar{x}$ AB	$\bar{x}$ A
		EM1	EM2	EM3		
160	427	281.45	282.57	281.55	281.86	282.27
	548	281.98	283.36	282.73	282.69	
$\bar{x}$ AC		281.72	282.97	282.14		
120	427	201.72	203.32	202.67	202.57	200.46
	548	200.59	196.41	198.03	198.34	
$\bar{x}$ AC		201.15	199.87	200.35		

102	427	170.25	206.29	196.58	191.04	185.53
	548	191.02	169.71	179.29	180.01	
x̄ AC		180.64	188.00	187.94		
x̄ BC	427	217.80	230.73	226.94	225.16	x̄ B
	548	224.53	216.50	220.02	220.35	
x̄ C		221.17	223.61	223.48		
Average					222.75	

	A*	B^{ns}	AB^{ns}	C^{ns}	AC*	BC**	ABC**
F test	0.00	0.33	0.59	0.22	0.01	0.00	0.00
LSD 0.01	12.28	11.07	19.17	3.17	4.49	4.48	7.76
LSD 0.05	16.40	16.77	29.05	4.29	7.36	6.07	10.52

Fertilization and the interaction of fertilization with treatments significantly influenced the differences in yield ($p<0.05$). Statistically, hybrids had the lowest grain mass at fertilization with 102 kg ha^{-1} (185.53 g). Hybrids and treatments did not have significant differences in the weight of 1000 grains, while the interaction of hybrids and treatments had a statistically significant effect on the differences in the weight of 1000 grains. The highest mass of 1000 grains was determined in the interaction of hybrid ZP 427 with both treatments (216.50-220.02 g).

The average yield of maize grains was 3.57 t ha^{-1} (Table 2). Statistically significant differences in yield were determined under the influence of fertilization and the interaction of fertilization with treatments ($p<0.05$). The treatments had a statistically highly significant influence, while the hybrids did not show a statistically significant influence on the grain yield. The highest yield was with fertilization with 120 kg ha^{-1} (3.77 t ha^{-1}), which was statistically significantly higher only in relation to fertilization with 160 kg ha^{-1}. The highest yield was in the treatment EM3 4.00 t ha^{-1}, which is statistically highly significant only in relation to the variant without treatment.

Table 2. The height of the yield of maize grains (t ha^{-1})

N kg ha^{-1} (A)	Genotypes (B)	Treatments (C)			x̄ AB	x̄ A
		EM1	EM2	EM3		
160	427	2.10	3.10	3.38	2.86	3.21
	548	3.41	3.65	3.63	3.56	
x̄ AC		2.75	3.38	3.51		
120	427	3.41	3.12	4.75	3.75	3.77
	548	2.99	3.73	4.27	3.66	
x̄ AC		3.20	3.43	4.49	3.71	

102	427	3.49	4.05	4.00		3.85	
	548	3.07	3.59	4.02		3.56	3.70
x̄AC		3.28	3.82	4.01			
x̄ BC	427	3.00	3.43	4.03		3.54	
	548	3.15	3.66	3.97		3.60	x̄ B
x̄ C		3.08	3.54	4.00			
Average						3.57	
	A*	B^ns	AB*	C**	AC*	BC*	ABC
F test	0.07	0.54	0.11	0.00	0.20	0.14	
LSD 0.01	0.54	0.41	0.52	0.34	0.60	0.66	
LSD 0.05	0.45	0.62	0.74	0.47	0.82	0.96	

Based on the obtained results, it can be said that by using EM Active as a biostimulator, stable yields can be achieved even with a reduced amount of mineral nitrogen. Also, the consequences caused by unfavorable abiotic influence can be mitigated. Taking into account the fact that maize is grown on the largest areas in the natural irrigation system, such research is certainly of great importance. Dry conditions in 2017 had a negative impact on the absorption of mineral substances from the soil, which was reflected in the grain yield. The protein content in maize kernels is on average 10-13% (Radosavljevic et al., 2020), while Ballesta and Lioveras (1996) determined that the nitrogen content in maize kernels ranges from 1.08% to 1.39%.

Nitrogen in the grain originates from nitrogen taken from the soil during grain filling and from nitrogen transported from the vegetative organs into the grain. The average nitrogen content was 1.40% (Table 3). All investigated factors and their interactions had a statistically significant effect on the nitrogen content. A statistically significant difference was found between the treatments, with the highest protein content found in the control variant.

Table 3. Nitrogen content in maize grain (%)

N kg ha-1 (A)	Genotypes (B)	Treatments (C)			x̄ AB	x̄ A
		EM1	EM2	EM3		
160	427	1.34	1.28	1.29	1.30	
	548	1.46	1.41	1.35	1.41	1.35
x̄ AC		1.40	1.34	1.32		
120	427	1.39	1.45	1.25	1.36	
	548	1.46	1.48	1.46	1.47	1.41
x̄ AC		1.42	1.47	1.35		
102	427	1.85	1.38	1.28	1.50	
	548	1.30	1.35	1.45	1.37	1.44
x̄ AC		1.58	1.36	1.37		

x̄ BC	427	1.52	1.37		1.27	1.39	x̄ B
	548	1.41	1.41		1.42	1.41	
x̄ C		1.47	1.39		1.35		
					Average	1.40	
	A**	B**n	AB**	C**	AC**	BC**	ABC**
F test	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LSD 0.01	0.02	0.01	0.02	0.03	0.06	0.05	0.08
LSD 0.05	0.02	0.02	0.03	0.04	0.08	0.06	0.11

Conclusion

Based on the obtained results, it can be concluded that both treatments with effective microorganisms would have a significant impact on the weight of 1000 grains and the yield. The greater effect of application is in variants with a smaller amount of mineral nitrogen. In general, it can be concluded that it is desirable to introduce the application of effective microorganisms as a supplementary measure.

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COBALT FERTILIZATION IN ORDER TO PROMOTE NITROGEN FIXATION IN ANNUAL FORAGE LEGUMES

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Abstract: The work aimed was to analyze the importance and possibilities of using cobalt for fertilizing annual forage legumes. Cobalt in small concentrations in the soil has a positive effect on the processes of nodulation and symbiotic nitrogen fixation. Therefore, its addition as fertilizer, especially on acidic soils, has a positive effect on the growth, yield, and quality of leguminous plants. Optimum concentrations of cobalt in the soil solution vary depending on the plant species and range from 8 to 50 ppm. Higher concentrations of cobalt in most annual forage legumes result in a toxic effect.

Keywords: cobalt, legumes, nitrogen fixation, nodulation

Introduction

Cobalt is an element necessary for normal leaf development, inhibition of ethylene biosynthesis, stimulation of alkaloid biosynthesis, processes of photosynthesis, plant respiration, etc. (Farooq et al., 2012). Cobalt fertilization is of particular importance for legumes due to its important role in the processes of nodulation and symbiotic nitrogen fixation (Tomić, 2017). Nodulation and nitrogen fixation in leguminous plants, especially on acid soils, depend to a large extent on the availability of cobalt in plants because it is an essential component of several important enzymes (Palit et al., 1994). Various studies indicate that seed vigor, nodule development, and nitrogen content in legumes depend on cobalt content in the soil and rhizosphere (Akbar et al., 2013). Optimum cobalt supply to plants contributes to enhanced nitrogen fixation in all *Rhizobium* species (Collins and Kinsela, 2011). Therefore, with a lack of cobalt in the plant, the organic production of legumes decreases (Vukadinović and

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Lončarić 1997). The aim of the work was to analyze the importance of cobalt fertilization for the process of nitrogen fixation and the productivity of one-year forage legumes, as well as the possibilities of cobalt application.

Effect of cobalt on nodulation and nitrogen fixation

Cobalt is the central atom in the porphyrin structure of cobalamin coenzyme, it is essential for nodulation and development of bacteroids (Akbar et al., 2013). It is a component of vitamin B12, which is included in the composition of enzymes and coenzymes important in the process of nitrogen fixation in leguminous nodules (Palit et al., 1994). Cobalt affects nitrogen fixation through different enzyme systems such as ribonucleotide reductase and methylalonyl coenzyme A mutase (Das, 2000). According to Farooq et al. (2012) several enzymes are activated by cobalt ions: E. coli acetylornithinase, Methanobacterium thermoautotrophicum cyclic 2,3-diphosphoglycerate hydrolase, α-d-mannosidase. However, a high concentration of cobalt in plants is also undesirable because it adversely affects the physiological and biochemical functions of the plant (El-Sheejh et al., 2003). The toxic effect of cobalt on plant morphology is manifested in the form of chlorosis, leaf drop, pale leaf nervure and reduced plant growth (Palit et al., 1994). Cobalt absorption on acidic soils is difficult because it is blocked by numerous other elements (Tomić, 2017).

Application of cobalt in annual forage legumes

The addition of cobalt to the soil during the production of forage pea (*Pisum sativum* L.) in the amount of 10-20 g ha^{-1} in the form of cobalt chloride ($CoCl_2 \cdot 2H_2O$), especially in combination with nitrogen fertilization, effected increasing the number of nodules per plant, the content of cobalt in nodules and shoots, nitrogen content in shoots and stem, flowers number per plant, pods number per plant, thousand grains mass, shoot length, root length, dry matter content in shoots and roots, yield and seed germination (Akbar et al., 2013). Bakken et al. (2004) found that there was no significant positive correlation between cobalt content and nitrogen content in the plant when cobalt was applied at a concentration of less than 0.02%. According to Nadia (2006), the application of cobalt in the amount of 8 ppm affected the increase of nodulation and the number of effective nodules in peas. The authors state that cobalt in the amount of 16 ppm influenced a significant increase in the number and mass of nodules, nitrogen concentration in nodules, leghemoglobin content, total biomass production, and seed yield compared to untreated varieties.

Pattanayak et al. (2000) stated that seed treatment with cobalt in a concentration of 0.008 mg g^{-1} of seed together with *Rhizobium* inoculation in cowpea (*Vigna sinensis* L.) influenced a significant increase in the total and number of effective nodules per plant, the mass of effective nodules per plant, the accumulation of dry matter in plants, number of pods per plant and seed yield per unit area. The authors state that cobalt is an essential element for nodulation and fixation of atmospheric nitrogen in cowpea. Similar results are indicated by Mathur et al. (2006), according to which the treatment of cowpea seeds with cobalt-nitrate in concentrations of 250 and 500 mg kg^{-1}, influenced a significant increase in the number and mass of effective nodules per plant, which was also reflected in an increase in the dry yield matter, of pod number per plant, thousand grains weight and total seed yield. The use of cobalt in cowpea significantly increased the number of nodules, their mass and nitrogenase activity, reducing ethylene production (Jain and Nainawatee, 2000). The application of cobalt in a concentration of 8 ppm over the soil in the form of irrigation influenced a significant increase in the number of nodules per plant, mass of nodules per plant, mass of dry matter of nodules, nitrogenase activity, plant height, number of branches and leaves per plant, leaf area, root length, dry matter of shoots and roots (Nadia, 2012).

Applied in the amount of 0.21 kg ha^{-1} cobalt in the form $CoSO_4 \cdot 7H_2O$ on the soil with 0,03 ppm of cobalt influenced the increase of plant height, number of branches and leaves, leaf area index, dry matter of shoots as well as the yield of peanut pods (*Arachis hypogaea* L.) (Basu, 2006). Banerjee et al. (2005) indicate that cobalt applied in the amount of 0.2 kg ha^{-1} in peanuts influenced the increase of plant height, branches and leaves number, leaf surface index, shoot dry matter, leghemoglobin content, and pod yield. According to Nadia et al. (2012) the application of cobalt in the amount of 8 ppm together with Mo in the form of an irrigation solution affected a significant increase in the dry matter yield of peanuts, especially when it was applied together with nitrogen in the form of urea, ammonium nitrate or ammonium sulfate. Cobalt fertilization also increased the number of nodules per plant, the mass of nodules per plant, the dry matter of nodules, their nitrogenase activity, the number of pods per plant, the mass of pods per plant, the content of oil in seeds and the yield of seeds per plant.

Threshold values for soil cobalt toxicity in beans range from 26 to 72 ppm (Chatterjee et al., 2006). Balachander et al. (2003) indicated that cobalt and molybdenum at a concentration of 50 ppm influenced a significant increase in plant height and biomass production of beans. Fertilization and seed treatment with cobalt increased nodulation, nitrogen fixation, uptake of nutrients, growth and yield of mango bean (*Phaseolus mungo* L.) (Pattanayak et al., 2000).

Cobalt in concentrations higher than 5 to 20 ppm influenced a significant increase in the number of nodules, plant height, number of stems, pod number per plant, dry matter of plants, number of seeds per plant, and seed yield of broad bean (*Vicia faba* L.) (Hala, 2007). An increase in the content of nitrogen, phosphorus and potassium, as well as the content of cobalt, iron, manganese, zinc and copper in the roots, shoots and seeds was also recorded during treatments with cobalt. According to Abdel-Moez and Nadia (2002), a good supply of bean plants with cobalt influenced the better formation of nodules on the roots and the fixation of atmospheric nitrogen by microorganisms that influenced the increase of nitrogen content in the plants.

The application of cobalt in the amount of 0.9 mg $CoSO_4{\cdot}7H_2O$ per pot with 5-8 kg of soil affected the increase in the mass of nodules on the main root of blue lupine (*Lupinus angustifolius* L.) (Dilworth et al., 1979). The number of bacteroids and the content of leghemoglobin in the nodules were directly dependent on the content of cobalamin. Nitrogen fixation activity depended on both cobalt status and cobalamin content in plants. 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.

Cobalt in the form of $CoCl_2$ up to a concentration of 50 mg kg^{-1} of soil had a positive effect on the growth of plant organs of soybean (*Glycine hispida* Max.) and the adsorption of nutrients from the soil. Higher concentrations of cobalt than this had a toxic effect on plants and led to less intensive growth (Jayakumar and Jallel, 2009).

Conclusion

Cobalt is a necessary element that, applied in small amounts, especially on acidic soils, can greatly influence the increase in the number of nodules per plant, mass of nodules per plant, dry matter of nodules and their nitrogenase activity in annual forage legumes. Thanks to this, fertilization with cobalt in the form of $CoCl_2{\cdot}7H_2O$ or $CoSO_4{\cdot}7H_2O$ had an indirect effect on many other processes in plants, affecting the increase in root length, plant height, nitrogen content in shoots and stem, number of branches and leaves, shoot dry matter, leaf surface index, leghemoglobin content, number of flowers per plant, number and yield of pods per plant, thousand grains weight, seed yield, seed quality and seed germination. The optimal concentrations of cobalt in the soil solution differed depending on the plant species and ranged from 8-50 ppm. Concentrations higher than those indicated in most annual forage legumes had a toxic effect.

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LIGNIFICATION AS THE MAJOR FACTOR LIMITING RED CLOVER DM AND NDF DIGESTIBILITY

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Abstract: NDF is a good indicator of fiber content in forages, but on the other hand NDF digestibility gives more accurate estimates of total digestible nutrients. The aim of this study was to evaluate two red clover cultivars for NDF digestibility and DM digestibility depending on the stage of the development - mid bud stage, early bloom and mid bloom, harvesting in three different cuts. Results of this study showed that lignin content increased with plant maturation in all three cuts. The highest lignin content was determined in the third cut ranged from 5.87 to 5.92% and 5.56 to 6.53% in DM of K 39 and K 32 red clover cultivars. Intensive lignifications of cell wall in the third cut influenced the highest decreasing of NDF digestibility.

Keywords: digestibility, lignin, red clover, stage of development

Introduction

Red clover is one of the most important leguminous plants grown in the temperate climate zone. It grows well on different types of soil, has the ability to fix atmospheric nitrogen and it is characterized by high nutritive value for ruminants (Leto et al., 2004).

There are many factors that impact and affect forage nutritive value. Plant maturity is the largest factor in limiting fiber digestibility (Buxton, 1996). This is due to the rapid accumulation of lignin in cell walls, as the plant matures (Jung, 1989). The cell wall is made up of different components whose interactions determine its structure and function. The main element that plays a leading role in regulating cell wall degradation is lignin with additional support from the cell wall components, due to the particular linkages that occur between cell wall constituents (Moore and Jung, 2001). However, harvesting forages at optimum stage of maturity is important to maximize both yield and quality including NDF (Neutral Detergent Fiber) digestibility. The indigestible fraction of NDF is

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a major factor affecting the utilization of carbohydrate sources as it varies greatly and may exceed more than one half of the total NDF in the rumen (Varga, 2006). Therefore, knowing information regarding forage digestibility and NDF digestibility is critical as it allows producers the opportunity to allocate higher digestible forages to higher producing cows and accordingly plan harvesting.

The aim of this study was to evaluate two red clover cultivars for NDF digestibility and DM digestibility depending on the stage of the development harvesting in three different cuts.

Materials and methods

This experiment was carried out at the experimental field of Institute for forage crops Kruševac, situated in central Serbia. Two cultivars of red clover - diploid *cv* K 39 (2n) and tetraploid *cv* K 32 (4n) were evaluated at three stages of development in the first, second and the third cut. The first studied stage of development was mid bud (MB), the second was early bloom (EBL) and the third was mid bloom (MBL).

NDF was determined according to the method by Mertens (2002). Lignin was determined as the residue insoluble in 72% (w/w) sulphuric acid, applying the method of Van Soest and Robertson (1980). The proportion of lignin in total NDF (L, % of NDF) was calculated as the percentage of lignin in the total NDF content. Two stage pepsin-cellulase method was used for *in vitro* DMD (Dry Matter Digestibility) according to the method by De Boevar et al. (1986). *In vitro* NDFD (NDF Digestibility) was determined according to the method by Riveros and Argamentaria (1987).

Experiment was established as a randomized complete block design in three replications, with factorial arrangements of two main factors (2 red clover cultivars x 3 stages of development) in all three cuts, separately. The data were processed by the analysis of variance in a randomized block design (ANOVA, Stat. Soft. STATISTICA 6). The significance of differences between arithmetic means was tested by Tukey test ($p < 0.05$).

Results and discussion

The content of NDF and lignin, as well as NDFD and DMD of two red clover cultivars at three stages of development harvested in three cuts are presented in the Table 1.

The NDF content in forages is the best single indicator of the feed intake potential in ruminants and a useful and reliable tool in the meals formulation for high-producing dairy cows. The results of this study showed that significantly changes in NDF content was observed in dry matter of diploid red clover cultivars, especially during the second and the third cuts. The content of NDF in dry matter of diplid red clover ranged from 40.36 to 49.54% of DM in the second cut and from 41.11 to 46.22% of DM in the third cut ($p< 0.05$), respectively. The results obtained in the investigation conducted by Buxton et al. (1985) showed that with advancing red clover development there was not a significant decline in quality as well as some other perennial legumes. Similar results were obtained in this study in dry matter of tetraploid red clover cultivar, we assume due to higher leaves proportion. Van Soest (1994) indicated that during the spring, high temperatures in interactions with the advancing of plant development lead to a much faster decline in quality than during the summer. Plants grown in spring can be characterized by very high quality if they are harvested early, but delaying harvesting can have a very negative effect on quality. In the late summer temperatures do not increase linearly and the advancing in plant development leads to a slow decline in quality (Buxton, 1996), which was confirmed in this study.

Table 1. Content of NDF, lignin, NDFD and DMD of red clover cultivars

			K 39			K 32	
Cut		MB	EBL	MBL	MB	EBL	MBL
I	NDF, %	38.48^{e}	45.54^{c}	44.54^{b}	40.09^{de}	41.00^{cd}	47.09^{a}
	Lignn, %	3.50^{c}	5.21^{a}	5.43^{a}	4.46^{b}	4.48^{b}	5.59^{a}
	L, % NDF	9.12^{b}	12.26^{a}	12.19^{a}	11.13^{a}	10.93^{a}	11.89^{a}
	NDFD,%	62.77^{a}	53.37^{b}	44.89^{c}	54.11^{b}	54.78^{b}	41.70^{d}
	DMD, %	83.55^{a}	79.56^{b}	76.74^{c}	80.60^{b}	80.21^{b}	74.30^{d}
II	NDF, %	40.36^{c}	44.62^{b}	49.54^{a}	45.28^{b}	44.15^{b}	45.27^{b}
	Lignn, %	4.36^{c}	4.86^{bc}	5.81^{a}	5.11^{abc}	5.39^{ab}	5.61^{ab}
	L, % NDF	10.80	10.92	11.73	11.29	12.20	12.39
	NDFD,%	57.88^{a}	49.77^{b}	51.32^{b}	51.72^{b}	36.78^{d}	47.27^{c}
	DMD, %	81.12	79.87	78.73	79.95	74.97	79.85
III	NDF, %	41.11^{c}	43.17^{b}	46.22^{a}	43.36^{b}	40.16^{c}	43.31^{b}
	Lignn, %	5.87^{ab}	5.92^{ab}	5.97^{ab}	5.56^{b}	6.08^{ab}	6.53^{a}
	L, % NDF	14.28^{ab}	13.71^{ab}	12.90^{b}	12.84^{b}	15.15^{a}	15.07^{a}
	NDFD,%	60.07^{a}	50.58^{b}	40.72^{c}	60.52^{a}	51.70^{b}	35.22^{d}
	DMD, %	84.77^{b}	82.05^{c}	78.44^{d}	87.90^{a}	83.08^{c}	75.80^{e}

NDF – Neutral Detergent Fiber; L – Lignin; NDFD – Neutral Detergent Fiber Digestibility; DMD – Dry Matter Digestibility; MB – Mid Bud stage of development; EBL – Early Bloom stage of development; MBL – Mid Bloom stage of development; Different letters in the rows denote significantly different means (p< 0.05) between the stages of development and cultivars

With red clover growth and development, lignin content increased in both investigated red clover cultivars. The highest value of lignin content was recorded in the MBL stage of development in all three cuts (Table 1). The least intensive changes with the plant growth and development were determined in the third cut of diploid red clover cultivar, and in the second cut of tetraploid red clover cultivar. In addition to being undegradable itself, lignin makes it difficult to break down the cellulose and hemicelluloses that could be used. This is the main reason why animals use less energy from the plants harvested in later stages of development. The main factors affecting the NDF digestibility is the degree of structural carbohydrates lignifications – the proportion of lignin in the total NDF. The results obtained in this study showed that proportion of lignin in total NDF increased with plants growth and development, except in the third cut of diploid red clover cultivar (Table 1). Although, the highest degree of fibre lignifications was found in the third cut of both investigated red clover cultivars. The degree of NDF lignifications in the third cut ranged from 14.28 to 12.90% of NDF in the DM of diploid red clover cultivar, and from 12.84 to 15.07% of NDF in the DM of tetraploid red clover cultivar.

As the result of intensive lignifications process NDF digestibility declined in both red clover cultivars harvested during all three cuts. The highest decreasing of NDF digestibility was observed in the third cut by 32.22% (relatively) in the diploid red clover cultivar and by 41.81% (relatively) in the tetraploid red clover cultivar, respectively regarding to the advancement of stage of red clover development in each cuts. On the other hand, the smallest decreasing of NDF digestibility was observed in the second cut (Table 1). DM digestibility decreasing with red clover growth and development due to increased concentration of the cell wall in the stem and due to decline and reduction of leaf-to-stem ratio was confirmed in this study. The results showed that the highest DM digestibility was in the third cut, although this period of vegetation was very dry and warm. Red clover harvested in the second cut was characterized by the similar values of DMD (ranged from 81.12 to 78.73% in diploid red clover cultivar and from 79.95 to 79.85% in tetraploid red clover cultivar). Lower DMD determined in the second cut than in the two others might be explain by a favourable conditions for intensive biosynthesis of structural carbohydrates and lignin and probably an unfavourable leaf-to-stem ratio. Taylor and Quesenberry (1996) pointed out that tha two most significant quality parameters are crude protein concentration and DMD. The value of both these parameters generally decrease with growth and development in all perennial legumes as a result of the leaf-to-stem ratio declining and intensive lignifications process.

Conclusion

Ruminants require forage NDF in the diet to maintain rumen function and maximize productivity, but also NDF digestibility is a key determinant of the nutritive value of a diet. The highest increasing of NDF content was observed in the second cut of diploid red clover cultivar, from 40.36 to 49.54% of DM. Lignin content increased in all cuts of both red clover cultivars, whereas the highest values were in the third cut. At the same time, the lowest NDF digestibility was observed at the mid bloom stage of development in the third cut for *cv* K 39 and *cv* K 32, 40.72 and 35.22%, respectively. Based on the obtained results we can recommend the use of red clover for ruminant nutrition at the mid bud stage of development.

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STRATEGIES FOR OPTIMIZED FERTILIZER USE: IMPLICATIONS FOR ENERGY EFFICIENCY AND CROP PERFORMANCE

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Abstract: The analysis underlines the crucial role of optimized fertilizer use in modern agriculture, which improves crop performance and energy efficiency. Efficient fertilizer management, including precision agriculture, is essential for sustainability. Data shows a 20% increase in wheat yields and up to 25% increase in soybean yields compared to conventional methods. Energy savings of up to 30% were also found in maize cultivation, 27% in sunflowers and 23% in sugar beet. Further research is essential to understand the long-term impact and scalability of optimized fertilization practices, with interdisciplinary collaboration driving innovation for a more sustainable agricultural future.

Keywords: fertilizer optimization, crop performance, energy efficiency, sustainable agriculture, precision agriculture

Introduction

In modern agriculture, the optimization of fertilizer use plays a crucial role in increasing crop yield and energy efficiency (Shariff et al., 2022). This shift in strategy is not only a response to the increasing demand for food, but also a crucial step towards sustainable agricultural practices that protect the environment while ensuring food security. With the global population expected to reach 9.7 billion people by 2050, the need for productive and sustainable agricultural systems is more urgent than ever (Rockstroem et al., 2017).

Sustainable agricultural practices are crucial as the sector faces the challenge of increasing food production by 70% by 2050, amid dwindling arable land and the effects of climate change (Pretty et al., 2018). Efficient fertilizer management is key to reducing reliance on chemical inputs, minimizing environmental impact and improving overall sustainability.

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Research has highlighted the complex relationship between agricultural practices and environmental sustainability, emphasizing holistic approaches that balance productivity and ecological health (Gascoigne et al., 2017). Optimizing fertilizer use is critical to reducing the environmental impacts associated with the overuse and mishandling of chemical fertilizers. Furthermore, energy efficiency in agriculture is about minimizing energy input while maximizing output, which makes precision agriculture technologies invaluable. With the help of soil sensors and data analysis, precision agriculture enables customized nutrient application that improves crop performance and reduces environmental risks (Shariff et al., 2022).

Optimized fertilizer use is essential to address the global challenges of food security, sustainability and energy efficiency in agriculture (Zanet al., 2016). In addressing the complexities of modern agriculture, the adoption of sustainable fertilizer practices will be critical for a resilient and environmentally conscious food system (Devi et al., 2023).

Materials and methods

The methodology used in the study investigates the impact of optimized fertilizer use on energy efficiency and crop performance through a comparative analysis of different fertilizer management strategies, including chemical, organic and biological fertilizers. It highlights the role of precision agriculture technologies, such as soil sensors, satellite imagery and data analytics, for monitoring and optimizing fertilizer use, drawing on the methodology of research by Rockstroem et al. (2017) and Pretty et al. (2018). The selection of studies for analysis is based on rigorous criteria, focusing on research published from 2000 onwards and considering a broad geographical scope to ensure a comprehensive understanding of global agricultural practices. The analytical approach involves synthesizing quantitative data from these studies, using statistical methods and meta-analysis to compare the results of different fertilization strategies. Among other things, the efficiency of nutrient use, crop yields, energy consumption and environmental impact are examined. The methodology also considers the ethical use of the data and acknowledges potential limitations, such as the variability of study designs and the challenges of applying the results in different contexts. These insights were gained through extensive focus groups, which provide different perspectives and ensure a holistic understanding of the topic.

Results and discussion

The literature on optimized fertilizer use encompasses different perspectives, methods and insights, with precision agriculture techniques at the core. Rockstroem et al. (2017) show the effectiveness of precision agriculture in improving nitrogen use efficiency and reducing environmental impacts. Conversely, Shariff et al. (2022) emphasize yield and quality improvements with controlled-release fertilizers, but also acknowledge economic challenges. Biofertilizers and organic amendments are emerging alternatives, as shown by Gavrilovic et al. (2023), who highlight their benefits in reducing emissions and improving soil health. Environmental impacts, such as greenhouse gas emissions, are addressed by Dyer and Desjardins (2003) and Lal (2004), who advocate optimized fertilizer use and conservation agriculture. Despite some disagreement, there is a consensus on the potential of precision agriculture and biofertilizers, which underlines the need for integrated approaches that take into account environmental, economic and social factors. To fully exploit the potential of optimized fertilizer use in sustainable agriculture, the gap between research and practice needs to be bridged (Kim et al., 2018; Michalik and Wandzik, 2020).

The results summarized from the literature and research findings show that the integration of precision agriculture and sustainable fertilization practices significantly increases crop yields and energy efficiency in different agricultural contexts. These results highlight the multiple benefits of optimized fertilizer use, including improved crop performance and reduced energy consumption.

Precision agriculture techniques, such as soil sensors and satellite imagery for targeted fertilizer application, have been shown to significantly increase crop yields. For example, Rockstroem et al. (2017) report a 15-18% increase in wheat yields through precision agriculture compared to conventional methods. Shariff et al. (2022) also found that the use of controlled-release fertilizers can increase soybean yields by up to 28%.

Table 1. Average yield increases trough the use of precision farming techniques

Crop type	Yield increase trough precision farming	Yield trough conventional methods
Wheat	+20%	Baseline
Soybean	+25%	Baseline
Maize	+18%	Baseline
Sunflower	+24%	Baseline
Sugar beet	+19%	Baseline

Source: Authors

Table 1 shows the average percentage increase in crop yields using precision farming compared to conventional methods for wheat, soybean and maize. Precision farming results in a 20% increase in wheat yields, indicating its significant contribution to wheat production. Soybean yields even increase by 25 %, which illustrates the effectiveness of precision farming in soybean cultivation. Maize yields are also benefiting with an increase of 18%. Sunflowers and sugar beet have also seen significant improvements, with yield increases of 24% and 19% respectively. These results underline the positive effects of precision agriculture on various crops due to optimized nutrient supply and water use.

The data underscore the significant benefits of precision agriculture techniques in increasing crop yields in various crops. These results underline the potential of precision agriculture as a valuable tool for improving agricultural productivity and ensuring food security in modern farming.

Optimized fertilizer use, especially through precision agriculture, leads to significant energy savings as less synthetic fertilizer needs to be produced and applied. Dyer and Desjardins (2003) estimate that optimized nitrogen management can reduce energy consumption in maize cultivation by up to 28 %. Lal (2004) confirms these results and points out that energy savings can also be achieved through conservation agriculture practices that improve nutrient use efficiency.

Table 2. Energy savings through optimized fertilizer management

Crop type	Energy savings (%)
Wheat	25%
Maize	30%
Soybean	20%
Sunflower	27%
Sugar beat	23%

Source: Authors

Table 2 shows the energy savings achieved through optimized fertilizer management in wheat, maize and soybean cultivation. Wheat cultivation shows a 25 percent reduction in energy consumption, which illustrates the significant efficiency gains from optimized fertilization practices. Maize cultivation benefited even more, with a 30% reduction in energy consumption, underlining the significant impact of efficient fertilization strategies. Considerable energy savings of 20 % can also be achieved in soybean cultivation, which indicates the positive effects of optimized fertilizer management. In sunflower cultivation, energy consumption is significantly reduced by 27% through advanced nutrient management, while sugar beet cultivation saves 23% energy through more efficient phosphorus use and less over-fertilization. These findings underscore the effectiveness of optimized fertilization practices to improve energy efficiency in different crops.

The data underscores the importance of optimized fertilizer management in reducing energy consumption across crop types. These results highlight the potential for improved energy efficiency in agriculture through the adoption of more sustainable and resource-efficient fertilization practices.

Yield improvements and energy savings vary by crop type, climatic conditions and soil type. Stevanovic et al. (2021) show that maize yields vary with rainfall patterns in response to biofertilizers, indicating the influence of environmental conditions on nutrient uptake efficiency. Miao et al. (2021) and Devi et al. (2023) show how saline soils affect the effectiveness of nitrogen management strategies and emphasize the importance of soil health for fertilizer optimization.

As Zhang et al. (2023) show, the use of organic additives and biofertilizers on intensively farmed fields increases the yield and microbial diversity in the soil. This is in contrast to extensive farming systems, where initial fertility and biodiversity can reduce the relative impact of such practices.

Table 3. Yield improvement and soil health indicators by farming system

Farming system	Yield improvement (%)	Microbial diversity	Nutrient availability
Intensive	+22%	High	Improved
Extensive	+10%	Moderate	Baseline

Source: Authors

Table 3 provides a comparison between two different cropping systems - intensive and extensive - in terms of yield increase, microbial diversity and nutrient availability. The intensive cropping system shows a significant yield increase of 22% compared to the extensive system. The extensive system, on the other hand, shows a lower yield improvement with an increase of 10 %. The intensive cultivation system is characterized by a high microbial diversity compared to the extensive system. This indicates the presence of a wider range of microorganisms in the intensive system, which can have a positive effect on the health and resistance of the soil. In the intensive cropping system, nutrient availability is improved compared to baseline levels, suggesting that plants are likely to receive the necessary nutrients for optimal growth and development. In contrast, nutrient availability in the extensive system is unchanged compared to the baseline, indicating less efficient use of nutrients or limited access to the required resources.

This analysis suggests that the intensive cropping system has advantages over the extensive system in terms of yield increase, microbial diversity and nutrient availability. This may have a significant impact on the sustainability and productivity of agricultural systems, especially in the context of increasing demand for food and limited resources.

Conclusion

Precision agriculture techniques have significant potential to improve crop yields and energy efficiency in various agricultural sectors. Studies have demonstrated the effectiveness of precision agriculture in improving nitrogen utilization, reducing environmental impacts and increasing yields and quality. These results highlight the multiple benefits of optimized fertilizer use, including improved crop performance and reduced energy consumption.

The energy savings achieved through optimized fertilizer management, particularly in maize cultivation, are considerable. Research shows that optimized nitrogen management can lead to significant energy savings on farms, supporting conservation agriculture practices that improve nutrient use efficiency. These findings underscore the importance of sustainable fertilization practices in reducing energy use in agriculture. The variation in yield increases and energy savings between cropping systems highlights the influence of factors such as crop type, climate and soil conditions. The contrasting results between intensive and extensive cropping systems highlight the need for context-specific approaches tailored to different agricultural contexts to maximize the benefits of optimized fertilizer use.

In summary, the integration of precision agriculture techniques and sustainable fertilization practices is a promising way to improve agricultural productivity, energy efficiency and environmental sustainability. Further research is needed to understand the long-term impact and scalability of optimized fertilization practices in different agricultural contexts. Interdisciplinary collaboration and integrated management systems are crucial to fully exploit the potential of optimized fertilizer use in sustainable agriculture and to ensure food security and environmental protection amidst evolving agricultural challenges.

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DEVELOPMENT OF WHEAT STEAM UNDER CONTAMINATION WITH HEAVY METALS

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Abstract: Heavy metals are significant environmental pollutants because they represent potential metabolic inhibitors. A study was conducted where the content of heavy metals in the wheat steam was examined, as well as the height of the plants from the soil level to the top of the highest coot. The aim of the research was to determine the content and influence of heavy metals on steam development in the Pobeda and Ljiljana varieties. An experiment was set up in pots where two different concentrations of a mixture of heavy metals 250ppm and 500ppm were added under controlled conditions.

Key words: atomic absorption spectrophotometry, heavy metals, wheat steam, *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 enter 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 increasingly become 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 parent rocks. Also, irrational use of organic and mineral fertilizers can lead to soil contamination. Toxic metals are absorbed by the plant through the roots from the soil, and from the atmosphere through the leaves (Mickovski-Stefanović et al., 2012).

Knowing the factors that influence the behavior and bioavailability of heavy metals in soil is essential. In the past period, the concentration of zinc in some soils has increased, especially in industrialized countries as a result of inadequate environmental protection from factory pollution. The most

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important factors for the dynamics of Zn accumulation are pH-value, $CaCO_3$ content and mechanical composition of the soil. Plants grown on contaminated soil absorb and accumulate heavy metals in their aerial organs (Jakovljević et al., 1997).

Due to the rapid increase in the population of people on earth, the requirements for the quantity of food are increasing. However, increasing anthropogenic accumulation of heavy metals can reduce yield and product quality (Popović-Vukeljić, 2002).

The concentration of heavy metals in plants indicates the degree of contamination, but also the ability of different plants to accumulate metals from soil treated with sewage sludge. The study showed the mobility and transfer of heavy metals from sewage sludge soils to different wheat cultivars. The experiment was carried out to study the transfer of heavy metals to wheat grain, namely wheat varieties grown on soil without waste sludge (control) and soil with waste sludge. A very high concentration of all heavy metals was found in the wheat grain from the land with waste sludge. And that only with the two varieties TJ-83 and Mehran-89, while with the varieties Anmol and Abadgar this was not the case as pointed out by Jamali et al. (2008).

Uncertainty of elevated cadmium concentration causes a number of negative effects in plants, such as inhibition of growth, enzyme activity, photosynthesis and change in stomatal activity (Prasad, 1995). The toxic effect of cadmium is reflected through its affinity to the thiol group, as a result of which cadmium can block the functional groups of biomolecules, the result of which is the inhibition of the enzymatic activities of the plant's metabolic processes, such as photosynthesis, respiration, assimilation of nutrient elements and transport (Ferreira et al., 2002).

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). In one study, the growth and concentration of elements in wheat that grew on 5 different soils treated with heavy metals was monitored. Each soil was mixed with $CdCl_2$ or $ZnCl_2$. The decrease in the concentration of elements in the soil varied depending on the type of soil. Wheat yield was reduced in all soil types and varied by soil type (Hattori and Chino, 2001).

The accumulation of heavy metals in a wheat crop exposed to different pollutants was studied. These experiments lasted 18 years on two types of soil.

The subject of research was spring wheat. The increased degree of soil contamination affected the activation of enzymes in the leaves and roots of plants and increased the degree of antioxidant protection against heavy metal ions. The concentration of heavy metals cadmium, zinc, lead and chromium in wheat grains was significantly lower than in straw, while nickel and copper were more in the grain. The roots contained the most cadmium, nickel, zinc and copper. The pH values had a slight influence on the content of copper, lead and chromium in plants (Murzaeva, 2004).

In some parts of China, research has been conducted on how heavy metal contamination affects plants and how plants provide resistance. Plant growth was inhibited, plant structure was damaged, physiological and biochemical activities fell. The bioavailability of heavy metals depends on numerous factors, such as environmental conditions, pH, types of elements and types of plants. There are also studies on plant defense mechanisms against the toxic effects of heavy metals, such as combining heavy metals with proteins and enzyme detoxification. These are mechanisms for protecting plants from damage by heavy metals. There are two aspects to the interaction of plants and heavy metals here. On the one hand, heavy metals have negative effects on plants, but plants also have their own defense mechanisms against the harmful effects of heavy metals (Cheng, 2003).

The effect of chlorimuron oxide and cadmium on common wheat was investigated. The combined effect on wheat contributed to weaken the possibility of chlorophyll formation. Wheat was able to protect itself by increasing the activity of the antioxidant peroxidase enzyme. Thus, the content of degradable proteins and peroxidase activity represent biomarkers for the negative effect of chemicals on the plant (Wang and Zhou, 2006).

Material and working 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 the growth of the plant was carried out through experiments in the greenhouse of the Faculty of Agriculture in Zemun, where the conditions of 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 pots 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-$Zn(CH_3COO)_2$ x $2H_2O$, lead in the form of lead acetate $C_4H_6O_4Pb$ x $3H_2O$, chromium in the form of chromium trioxide-CrO3, copper in the form of copper sulfate-$CuSO_4$ and cadmium in the form of cadmium nitrate-$Cd(NO3)_2$ x $4H_2O$.
The following concentrations of solutions were used:
0 ppm (control), 100 ppm, 250 ppm

In addition to the content of heavy metals in the wheat stem, the height of the stem from the soil level was first measured. After that, the content of heavy metals in wheat stems was determined.

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-steams 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. A gentle boiling was performed for 2 hours. After cooling, 3 ml of H_2O_2 was added, and then boiling was performed for 15 minutes. The procedure with peroxide was repeated. After cooling, 2 ml of HClO4 was added and gentle 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 normal 50 ml vessels. The vessels 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.

Research results and discussions

Steam height

The Pobeda variety had an average height (42.40 cm), while the Ljiljana variety had a lower average height (39.66 cm). The average height of the steam per sample for the variety Pobeda varied from 34.00 to 50.60, and for the variety Ljiljana from 33.50 to 35.50 (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*
250 ppm	*34,00*	*1,8371*	*3,3723*
500 ppm	*50,60*	*1,0625*	*1,9503*
Average	*42,40*	-	-

Table 2: Stem hight, variety Ljiljana, 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*
500ppm	*33,50*	*3,3119*	*6,0795*
Average	*39,66*	-	-

At a mixture concentration of 250 ppm, the height of the steam in the variety Ljiljana was 35.50 cm, while in Pobeda it was lower and amounted to 34.00 cm. When contaminated with a mixture of metals with a concentration of 500 ppm, the variety Ljiljana had a stem height of 33.50 cm, and the variety Pobeda was slightly taller at 50.60 cm.

Differences in stress-induced effects of elevated concentrations of toxic metals indicate different tolerance of plants to metals (Zhao et al., 2017).

The content of heavy metals in the stem of wheat

The content of heavy metals in the varieties Pobeda and Ljiljana varies greatly (tables 3 and 4).

According to their average content in the wheat stem of the Pobeda variety, the investigated heavy metals can be arranged in the following order:

• zinc > cadmium > copper > lead > chromium

The lead content of the Pobeda variety in the wheat stem is on average 4.13 mg kg -1 and varies from 2.46 mg kg -1 (control) to 5.45 mg kg -1.

According to their average content in the wheat stem of the Ljiljana variety, the examined heavy metals can be arranged in the following order:

• zinc > cadmium > copper > lead > chromium

The lead content of the Ljiljana variety in the wheat stem is on average 4.25 mg kg $^{-1}$ and varies from 2.14 mg kg $^{-1}$ (control) to 6.28 mg kg $^{-1}$.

The results of statistical processing (analysis of variance) show that the experimental factor-concentration of the mixture of heavy metals for contaminating the soil had a very significant influence on the content of heavy metals in parts of wheat, which can be seen on the basis of the 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*
250 ppm	*49,10*	*5,45*	*1,75*	*5,54*	*9,12*	*49105,52***	*0,2678*	*0,3703*
500 ppm	*122,77*	*4,49*	*0,64*	*8,14*	*27,00*	*56428,15***	*0,6525*	*0,9023*
Prosek	*109,41*	*4,13*	*1,21*	*6,05*	*12,26*	-	-	-

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*
250 ppm	*79,13*	*4,33*	*1,23*	*5,41*	*17,31*	*58501,43***	*0,4087*	*0,5652*
500ppm	*203,47*	*6,28*	*1,15*	*6,39*	*34,87*	*87931,24***	*0,8808*	*1,2181*
Prosek	*104,20*	*4,25*	*0,99*	*5,48*	*17,52*	-	-	-

Conclusion

Vegetation experiments in pots were performed with different concentrations of heavy metal mixtures. Increasing the concentration of the metal mixture had a negative impact on steam height. Higher concentrations of heavy metals significantly reduced plant growth in both cultivars.

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INFLUENCE OF DIFFERENT SUBSTRATES ON THE QUALITY OF PEPPER SEEDLINGS (*Capsicum annuum* L.)

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Abstract: The paper shows the influence of different substrates on the quality of pepper seedlings. Based on the obtained results, it can be concluded that different substrates had an impact on the biological-morphological characteristics of pepper seedlings. The best results for most of the analyzed biological-morphological characteristics of pepper seedlings, according to the results obtained by the experiment, are related to the substrates "Glistenjak", "K-Potgrond H-Black 90%" and "K-TS1-White 100%", while the worst results were achieved on the examined substrates of "Coconut Cube". The mean value for all traits was recorded on the substrate "K- Seedling substrat".

Key words: pepper seedling, substrates, quality, *Capsicum annuum*.

Introduction

Paprika (*Capsicum annuum* L.) represents one of the most significant vegetable crops in Serbia. According to data collected by the Food and Agriculture Organization (FAO), worldwide, in the last 5 years, the areas cultivated with paprika have been increasing. In Serbia, however, this trend is reversed. The area dedicated to paprika cultivation decreased by 41% in 2020 compared to the year 2016, while the total production was reduced by a staggering 53% (FAO, 2022). The reasons behind this decline should be sought in the increasing production costs, lack of labor force, low yields, low market prices for paprika fruits, and so on.

Pepper production is most commonly carried out through seedlings. The seedling period is the time when the plant goes through the stages of germination and the first four to five stages of organogenesis. The overall yield, earliness, and fruit quality depend on the quality of the seedlings (Lazić, 1993). To avoid stress, seedling production with a protected root system is employed,

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using pots, containers, nutrient blocks, etc. The roots then bind to the substrate in which they grow and from which they are transplanted. They are collectively pulled out of pots (containers), minimizing potential damage to the root system.

The substrate plays a crucial role in the production of high-quality vegetable seedlings, and to a large extent, it determines success in production. The substrate is important because it is in this medium that delicate, young plants go through their most sensitive life phase (Zeljković et al., 2017).

In today's times, there is significant interest in the impact of different cultivation systems on food quality, especially regarding its effects on human health (Zdravković et al., 2010). The demand for organically produced food is steadily increasing. The reason for this lies in consumers' desire for food from reliable sources, where the production process is environmentally safe (air, soil, water), and the produced food product is of high quality (Burt et al., 2009; Domagala-Swiatkiewicz and Maciej 2012; Kazimierczak et al., 2014). Organic production represents an area of high potential, both in terms of increasing the areas under organically cultivated products and in terms of the added value that these products generate (Ivanović and Ivanović, 2016).

The aim of this study was to determine the effect of different types of substrate mixtures available on the Serbian market on the production quality of bell pepper seedlings (plant height, stem diameter, number of leaves, total plant mass, total number of flower buds and total root volume).

Materials and methods

The experiment was set up and conducted in a greenhouse with five treatments at the Novo Selo location, Vrnjačka Banja municipality. Each treatment included 20 plants arranged in columns according to the treatments. The chosen pepper variety is "Belinda". Pepper seeds were sown in plastic containers with 66 holes. When the seedlings were ready for transplanting, 14 days after sowing in the containers, young plants were transplanted into plastic cups measuring 10 × 10 cm, filled with 5 different types of substrates. Plants that were sown and germinated in identical substrates in containers were transplanted and grown until the end of the experiment. The technology for growing young papper plants followed the principles of organic plant production. The experiment concluded when the pepper plants reached the stage for permanent transplanting (62 days from seed sowing). Four different organically certified substrates and one non-organic substrate (Klasmann "TC1-White 100%", Klasmann "Potgrond H-Black 90%", "Glistenjak", "Coconut

Cube”, Klasmann “Seedling Substrat”) were tested in the experiment. This research determined the impact of different substrates (a total of five) on the quality parameters of papper seedlings, including plant height, stem diameter, leaf number, total plant mass, the number of flower buds per plant, and root volume. The collected data were analyzed using Microsoft Excel software, applying the Least Significant Difference (LSD) test. The LSD test is the simplest method for comparing the arithmetic means of the observed samples.

Results and discussion

Analyzing the height of young pepper plants, stem diameter, total number of leaves, mass of young plants, total number of flower buds per plant, and root volume, it can be clearly observed that based on the calculated LSD test at the significance levels of 0.05 and 0.01, all tested substrates ("Potgrond H-Black 90%", "Glistenjak", and "K-TC1-White 100%”, “K-Seedling substrat”, and “Coconut Cubes”) in this experiment had a statistically significant impact on pepper seedlings. Additionally, they differed significantly from each other (Table 1).

The substrates "Potgrond H-Black 90%", "Glistenjak", and "K-TC1-White 100%" had the best values for all analyzed quality parameters of pepper seedlings. In fact, the best results for the parameter of the total plant mass (31.35 g) and the total number of flower buds (9.6) were achieved by cultivating pepper seedlings on the substrate "Potgrond H-Black 90%". The best results for most other analyzed characteristics, such as plant height (30.08 cm), stem diameter (6.57 mm), total number of leaves (22.45), total plant mass (31.35 g), and total root system volume (24.2 cm^3), were achieved by cultivating pepper seedlings on the "Glistenjak" substrate. Meanwhile, plants grown on “Coconut Cubes” substrate showed the lowest values for all examined properties. The substrate with intermediate values for all tested quality parameters of pepper seedlings is the “K-Seedling substrat” (Table 1).

Table 1. Mean values for analyzed properties of peppers by treatments

Treatments/Properties	Plant height (cm)	Stem diameter (mm)	No. of leaves	Total plant mass (g)	Total No. of flower buds	Total root volume (cm^3)
Glistenjak	30.08a	6.57a	22.45a	12.07a	8.40b	24.2a
Coconut Cube	4.62c	1.84c	5.70c	0.15c	0.00d	0.25b
K-TC1-White 100%	27.85a	6.10a	21.95a	9.70b	8.30b	24.05a
Potgrond H-Black 90%	31.37a	6.52a	21.65a	12.23a	9.60a	23.00a

K- Seedling substrat	16.98b	4.33b	12.50b	2.42c	2.50c	6.55b
LSD (0.05)	5.49	0.80	5.58	2.46	1.08	8.92
LSD (0.01)	7.98	1.16	8.12	3.58	1.43	12.98

Values marked with different small letters in the column significantly differ at the P≤0.01 level according to the LSD test

Examining plant growth, some authors consider it essential to enrich the substrate with compost, zeolite, and NPK fertilizers to enhance the growth and development of seedlings (Marković et al., 1994). In our research, there was no additional enrichment of the substrate, and for most tested substrates, it was not deemed necessary. Furthermore, various studies, including those by Atif et al. (2016), Damjanović et al. (2005), Gutierrez-Miceli et al. (2007), Herrera et al. (2008), and Ugrinović et al. (2018), have confirmed the significant impact of substrates on the growth and quality parameters of seedlings. Proper substrate selection is crucial for growers to produce high-quality seedlings (Gutierrez-Miceli et al., 2007; Herrera et al., 2008; Morales-Corts et al., 2014; Atif et al., 2016; Ugrinović et al., 2018; Ugrinović et al., 2021). Many studies have also confirmed that the total number of leaves per plant depends on the nutritional regime of the substrate, water regime, and other ecological factors. Plants of the same age may have different leaf numbers depending on cultivation conditions (Marković et al., 1994; Damjanović et al., 2005; Kang et al., 2011; Ugrinović et al., 2018). The number of formed flower buds per plant is crucial for yield, with agro-ecological and genetic factors influencing this trait significantly (Kang et al., 2011; Huvelink, 2005; Zdravković et al., 2012; Ugrinović et al., 2018). The substrate mixture also has a considerable impact on the growth of the root system and its mass, as confirmed by Damjanović et al. (2005) and Ugrinović et al. (2018).

Conclusion

Different substrates used in this research have a significant impact on various parameters of pepper seedling quality. Plant height, stem diameter, leaf count, total plant mass, number of flower buds per plant, and root volume are parameters that varied significantly depending on the type of substrate used.

Substrates such as "Glistenjak," "Potgrond H-Black 90%," and "K-TC1-White 100%" proved to be effective in supporting plant height, stem diameter, and leaf count, while the “Coconut Cube” substrate showed certain challenges in these aspects. On the other hand, in parameters such as total plant mass, number of flower buds, and root volume, "Potgrond H-Black 90%," "Glistenjak," and "K-TC1-White 100%" stood out with high values.

These results emphasize the importance of selecting an appropriate substrate in the pepper seedling production process, as different substrate characteristics can directly influence plant development. Proper substrate selection can improve the growth and development of pepper plants, significantly impacting overall production.

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BREAD WHEAT FROM STRESS TO PRODUCTIVITY IN PURSUIT OF FOOD SECURITY IN RAPID CLIMATE CHANGE

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Abstract: The sample of 596 plants, in total, of bread wheat varieties Pobeda, Sara, Renesansa, and Pesma were tested for abiotic stress tolerance in eight environments, out of which six were under soil or/and atmospheric stress. Plant adaptive plasticity was followed by the variation of three phenotypic markers: grain weight/spike, spike length, and grain number per spike. A desirable genetic variation was recognized and singled out within the existing gene pool, to enhancing stress tolerance in wheat in order to face the challenges and contribute to food security in rapid climate changes.

Keywords: wheat, stress, tolerance, spike traits, food security

Introduction

Climate changes and global warming that have been speeding up in the last 65 years influence various aspects of human life, food production and security included (Abbas et al., 2022). Great efforts are put to meet these challenges, especially in classic and molecular plant breeding. A part of these efforts are directed in mitigation of stressful effects of degraded and naturally low productive soil, particularly in enhancing adaptive plasticity of wheat, as one of the staple food (Bhoite et al., 2023; Johanson et al., 2023). Genetics at the Faculty of Agriculture, University of Novi Sad, has been engaged, for more than twenty years, in research on behavior and adaptive plasticity of wheat in stressful conditions. The results are scientific knowledge, disseminated results, and training of young researchers (Petrović et al., 2003; Petrović et al., 2023).

The aim of this study is to get an insight into the adaptive plasticity of a complex sample of four bread wheat varieties exposed to a different kind and intensity of abiotic stress. To single out usable genetic variability within the existing genetic gene pool for the wheat tolerance increment to solonetz soil type and atmospherically caused stress.

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Materials and methods

A population of four wheat (*Triticum aestivum* L. subsp. *aestivum*) varieties, Pobeda, Sara, Renesansa and Pesma, from the wheat breeding programme of the Institute of Field and Vegetable Crops in Novi Sad (IFVCNS), was grown in four seasons at two localities. One locality was at the experimental field of the Faculty of Agriculture, UNS at the village of Kumane (K), exact Google map location 45°31'19.3"N 20°11'40.6"E, on the solonetz soil type (SN) of unfavorable high sodium and clay content in the Bt horizon (Belić et al, 2012). The other was, 35.32 km straight line distance to the SW from K, at the IFVCNS experimental fields in Rimski Šančevi (RS), 45°19'21.9"N 19°50'22.2"E, on the fertile chernozem soil (CH). Four seasons were chosen out of twenty years long multi genotype and environmental trial (MGET). The season 2004/05 was characterized by abundant precipitations, water excess (WE), and soil saturation. The season 2011/12 was a period of long-lasting drought, and temperature extremes (DTE). The season 2015/16 was very favourable (VF) with the wheat yield 20% higher than in previous ten years. The season 2019/20 was a normal, average season (NA) with wheat yield within multiyear average. That made eight environments (E_n). The total wheat sample consisted of 596 wheat plants. In E1, of combined stress sources (SN+WE) n=60 individual plants of four wheat varieties were tested; E2 (CH+WE) n = 60; E3 (SN+DTE) n=60; E4 (CH+DTE); E5 (SN+VF) n=120; E6 was the first non-stressful season (CH+VF) n=120; E7 (SN+NA) n=58, and E8 was the second non-stressful season (CH+NA) n=58. The phenotypic markers for plant discriminatory behaviour across the environments were SL - spike length (cm), GNS - grain number/spike, and GWS - grain weight/spike (g). Their values were standardized by z-scoring to common scale. The mean (μ) was substracted from observed values (x), and divided by the standard deviation [$z = (x-\mu)/\sigma$]. That way variables with the larger scale are prevented to dominate the analysis, and the contribution of variables measured in different units or with different variances is brought to more comparable level. The multiple discriminant analysis (MDA), and canonical discriminant analysis (CDA), was used. Since dependent variable (y), the stress absence (0), or presence (1) as input gave a binary outcome, the linear probability model (LPM) was utilized (Hair et al., 2010; Gomila, 2021).

Results and discussion

The effect of different stressful, and non-stressful environments on phenotypic variation of GWS, SL, and GNS, as discriminative phenotypic markers of choice revealed that solonetz soil type, of unfavourable physical and chemical properties, was of the primary stress source in the MGET. The atmospheric stress modulated the strength of that stress. The primary stress on chernozem soil type was atmospherically induced, where favourable soil type has alleviated the deleterious effects of water excess, but the drought and temperature extremes have taken the toll. The strongest stress, according to GWS and GNS averages, was on the SN+NA. The combination SN+WE had more stressful effect than SN+DTE due to unfavourable solonetz physical properties causing water lodging. Very favorable meteorological conditions in K had the stress effect like a water excess at the same locality, probably due to complex of abiotic and biotic influences. The depression of GWS and SL means in a meteorologically VF season was denoted in chernozem, too. Nevertheless, it should be kept in mind that the traits of individual plants were measured, meaning that the focus of the study was individual genotype behavior that could deviate from population reaction to environmental variation. The canopy thinning opened the vegetation space for the remaining plants to try to compensate the losses, as well as to develop resistance mechanisms. Hence, the losses were greater in the yield per unit area. Standardized mean values provide a closer insight into the environmental variation effects on phenotypic variability of studied spike traits. The SL was the least affected by stresses. Its reduction was notable in K, except in E3. For the GWS and GNS K locality was stressful in all the seasons, varying in intensity depending on the atmospheric conditions. In RS atmospheric stress left its toll on spike yield, and GNS in E4, where drought and temperature extremes hit the MGET (Table 1).

Table 1. Four seasons at two localities i.e. six stress and two non-stress environments, four wheat varieties, and three traits, GWS - grain weight/spike (g), SL - spike length (cm), and GNS - grain number/spike made the MGET

Soil type	Solonetz (Kumane)				Chernozem (Rimski Šančevi)			
Season / Trait	Water excess	Drought/ Temp. Ex.	Very favour.	Average season	Water excess	Drought/ T.E.	Very favour.	Average season
Seasonal mean values								
GWS	1.29	1.52	1.23	1.07	2.07	1.64	2.23	2.90
SL	7.37	8.63	7.63	7.59	10.25	9.37	9.07	9.34
GNS	35.48	38.82	30.85	27.90	44.60	33.31	49.15	56.60
Seasonal standardized mean values								
GWS	-0.6477	-0.3234	-0.7246	-0.9532	0.4298	-0.1630	0.7795	1.5683
SL	-0.8144	0.0245	-0.6374	-0.6648	1.0959	0.5100	0.3114	0.4952
GNS	-0.3413	-0.0685	-0.7208	-0.9626	0.4051	-0.5195	0.7775	1.3875

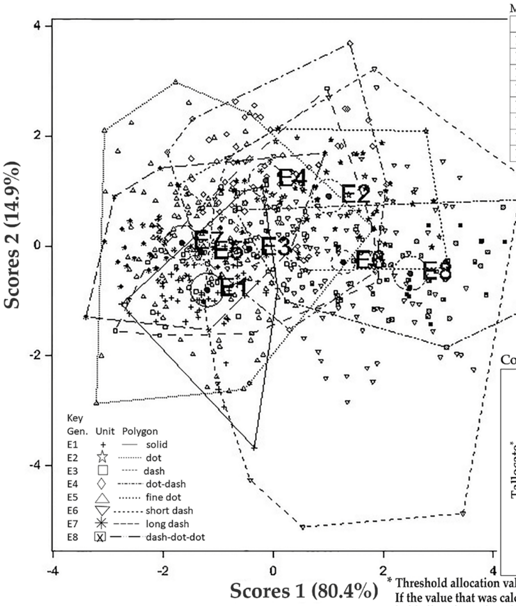
The LPM gave a first insight into eventual higher stress tolerance genetic variability existence within four tested wheat varieties. In a total sample of 596 plants in eight environments, the number of stressed plants was $n_0 = 418$, while in non-stress environments were $n_1 = 178$ plants. According to basic regression statistics, the overall correlation between GWS, SL, and GNS as stress/non-stress predictors and stress/non-stress outcome, brought out by Multiple R was 0.688, while coefficient of determination was $R^2 = 0.473$, indicating that about 50% of phenotypic variation across environments could be explained by joint variation of predictors in study. Though, residual SS was slightly higher than regression SS, the overall significance of the regression still stood, indicated that one or more predictors significantly influenced stress/non-stress plant's adaptive discrimination. Coefficients of LPM indicate that all three phenotypic markers significantly discriminated individual plant reaction to stress (Table 2).

Table 2. Linear probability model as a special case of multiple linear regression

Regression statistics		ANOVA					
			df	SS	MS	F.	F-sig.
Multiple R	0.687640758	Regression	3	59.03006	19.67669	177.007	6.598E-82
R Square	0.472849811	Residual	592	65.80886	0.111164		
Observations	596	Total	595	124.8389			

Coefficients of LPM					
	Coefficients		Standard Error	t Stat	P-value
Intercept	0.298657718	β_0	0.013657091	21.86832555	3.73573E-78
GWS (x_1)	0.261780485	β_1	0.030794126	8.500987584	1.53227E-16
SL (x_2)	-0.04922463	β_2	0.015560996	-3.16333406	0.001639652
GNS (x_3)	0.080482673	β_3	0.030191543	2.66573566	0.007891744

The average discriminant score ($\bar{d}$) as the mean value of individual LP $\hat{Y} = \beta_0+\beta_1x_1+\beta_2x_2+\beta_3x_3$) for stress ($\bar{d}_0 = 0.1574$), as well as, non-stress ($\bar{d}_1 = 0.6303$) environments and the number of unsatisfactory (stress) $n_0 = 418$, and satisfactory (no stress) $n_1 = 1$ binary Y, gave a cutoff between stress and non-stress environments based on LPM [$c = (n_0\bar{d}_0 + n_1\bar{d}_1)/(n_0+n_1) = 0.2987$]. A binary predictor outcome was established in respect to the c. The misclassification check was established comparing the binary input and the binary outcome, giving 79.9% concordance of binary classification based on soil and atmospheric conditions and binary classification based on phenotypic variability i.e. the discriminative power of GWS, SL, and GNS. That has left 20.1% of misclassified phenotypic variability worthy of further analysis in quest for higher stress tolerance variation. Mahalanobis distances (D2) were used to quantify the separation between observations and/or environmental means. The primary discrimination source was soil type on the first discriminative function. The secondary canonical variates were meteorological conditions. It was less discriminatory in K, than in RS, followed by D^2 that ranged in K from 0.157 (E5/E7) to 2.120 (E3/E7), while in RS D2 varied broader from 1.743 (E2/E6) to 9.753 (E4/E8). Threshold allocation value (Tallocate), determined the belonging of each individual genotype to an appropriate environment. The accompanying allocation table shows counts for that threshold allocations, observed in canonical space. Two behavioural patterns were observed. One is narrower adaptation, where individual plants adaptive reaction is limited to the stress presented in their environment. The other is broader adaptive reaction, which indicate that those plants could adapt to different kind of stress and/or different stress levels. In that second adaptive plasticity pattern, genotypes of particular interests are those who are coming from stressful, but are allocated to non-stressful environments, according to calculated threshold allocation values. This is an indication, based on discriminant analysis of the spike yield, SL, and GNS that enhanced tolerance to abiotic stress studied in this MGET could be found within an existing wheat inter and intra varietal variation (Graph 1).



Mahalanobis (D^2) quantifies intergroup distances

	E1	E2	E3	E4	E5	E6	E7	E8
E1	0.000							
E2	7.829	0.000						
E3	1.232	3.019	0.000					
E4	5.487	2.133	2.537	0.000				
E5	0.690	6.909	1.379	3.122	0.000			
E6	6.421	1.743	3.297	4.345	6.730	0.000		
E7	1.157	8.337	2.120	3.646	0.157	8.776	0.000	
E8	14.114	4.947	9.695	9.753	14.577	1.685	17.563	0.000

Discriminant scores for the environmental means

	1	2	3
E1	-1.1970	-0.7964	0.2547
E2	1.0108	0.9047	0.5006
E3	-0.4356	-0.0550	0.5756
E4	-0.1313	1.1964	-0.3619
E5	-1.3026	-0.1184	-0.2138
E6	1.2753	-0.2947	0.0166
E7	-1.6573	0.0587	-0.2082
E8	2.4927	-0.4969	-0.3862

Counts for allocation of 596 genotypes over eight environments

	Environments									
Tallocate*	Counts	E1	E2	E3	E4	E5	E6	E7	E8	Total
	E1	42	.	12	.	29	6	6	.	95
	E2	.	31	5	7	.	19	1	.	63
	E3	6	3	21	9	14	14	7	2	76
	E4	3	16	3	30	9	6	4	.	71
	E5	8	.	9	5	21	1	8	.	52
	E6	.	3	6	5	3	40	.	12	69
	E7	1	1	4	4	44	.	32	.	86
	E8	.	6	.	.	.	34	.	44	84
	Total	60	60	60	60	120	120	58	58	596

* Threshold allocation value Tallocate = $(\mu i + \mu j)/2$, where μi and μj are means of two environments; If the value that was calculated based on traits data i.e. discriminant score is greater than the threshold allocation value, than the genotype is assigned to the Ei, otherwise it is assigned to the Ej.

Graph 1. Canonical discriminant analysis of wheat four varieties population behaviour under six stress and two non-stress environments

Conclusion

A complex wheat varietal sample of 596 plants, subjected to six stressful and two non-stressful environments. GWS, SL, and GNS, were of discriminant value in DA. Potential higher tolerance to solonetz soil and atmospherically caused stresses were spotted within the existing gene pool. Further study would be required for deeper insight. The stress tolerant genotypes within the existing genetic variability could be of immediate use, as well as, for creating novel improved genetic variation in pursuit of food security in climate changes.

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SUSTAINABLE MANAGEMENT OF AGRICULTURAL RESOURCES IN MAIZE PRODUCTION IN BIH - H2020 SMARTWATER PROJECT

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Abstract: The increased need for smart management of agricultural resources resulted in the preparation and implementation of H2020 project SMARTWATER. This publication aims to present the main outcomes of SMARTWATER in three years of implementation (2021-2023), to encourage relevant target groups to participate in the action in 2024 and to promote smart management of agricultural resources. During project implementation different results were obtained. SMARTWATER team will continue with different twinning activities in 2024 aiming to promote smart agriculture practices, increase the competencies of scientists and young researchers and disseminate the project outcomes.

Keywords: field crops, irrigation, young researchers, sustainability, water

Introduction

In previous research, Bosnia and Herzegovina was characterized as a country with a good irrigation potential but relied on rainfed agriculture practices, which impose a need for smart management of land and water resources (World Bank, 2012; FAO, 2017). These and similar findings resulted in the preparation of a proposal and implementation of a new H2020 project titled SMARTWATER[2]. A project was launched on the 1st of January 2021. Main project topics include: 1) cloud-based smart technologies, 2) new generation of satellite remote sensing data, 3) water-energy-food nexus and 4) climate change impact on agriculture. Publications regarding these topics were prepared and published by SMARTWATER team during the implementation in scientific Journals and Conference Proceedings (Zenodo, 2024).

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

Within WP3, joint experimental studies are being conducted at two locations in BiH, with maize as the main crop and different irrigation and fertilization amounts as the main factor modalities. Irrigation and fertilization treatments in maize were investigated in some previous research (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's initial duration was 36 months but now it has been extended to 42 months (until June 2024). SMARTWATER consortium consists of six partners: UNI-BL, CIHEAM-IAMB, CSIC, ISA, SYS and UNSA[4] and implementation has been done through five Work Packages (WPs).

This publication aims to present the main outcomes of SMARTWATER project in three years of implementation (2021-2023), to encourage all relevant target groups to participate in the action in 2024 and to promote smart management of agricultural resources in Bosnia and Herzegovina and abroad.

Project implementation in 2021 - brief overview

The kick-off meeting was held in a hybrid form on the 27th of January 2021 and gathered all partners. Communication channels were established (official website and social media profiles on *Facebook*, *Twitter*, *LinkedIn* and *YouTube*). The UNI-BL team participated in the REA Cluster Event[5] (20.5.) and in the symposium AgroReS 2021[6] (27-28.5.). The 1st stakeholders' meeting was organized by UNSA (7.6.).

The 1st summer school "*Integrated approach for agricultural water management*" was organized in Trebinje (30.8.–3.9.). SMARTWATER project was presented in an interview with INTRASOFT International[7].

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

We participated in 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].

ISA organized the first advanced training course "*Advanced remote sensing technologies and tools for crop water requirements estimates and irrigation scheduling*" in Lisbon (27.9.–1.10). The 1st CATCHaCORN[10] students' competition was organized by UNSA (24.10.).

The 1st year of experiments was finished. Three master students started their course (1.10.). All project reports for 2021 were sent to EC. The dissemination was an ongoing activity to spread knowledge about the project and to gather and involve target groups in activities.

Project implementation in 2022 - brief overview

UNIBL joined the “*World Water Day*[11]*”* in Patras in Greece (22.5.) and the “*5th International Scientific Conference on Water*[12]*”* in Szarvas in Hungary (22-24.3.). SMARTWATER was presented in Trebinje at “*AgroReS 2022*[13]” (26-28.5.). ISA team joined the “ENCONTRO CIÊNCIA[14]” in Lisbon (16-18.5.).

UNSA team organized the 2nd stakeholders’ meeting (20.6.). ISA participated in the “*National Fair of Agriculture*” in Portugal (4-12.6.).

The 2nd summer school “*Smart technologies and best practices (technical and practical) for sustainable and environmentally efficient water management in agriculture*” was organized by UNSA in Sarajevo (18-22.7). SMARTWATER was presented at the “*International Symposium on Managing Land and Water for Climate-Smart Agriculture*[15]” held in Vienna, Austria (25-29.7.). UNSA team organized the first workshop on funding opportunities and proposal drafting in Sarajevo (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.).

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[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/
[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/

CSIC organized the 2nd advanced training course "*Use of innovative technologies and tools for collective and on-demand pressurized irrigation systems*" in Zaragoza (26-30.9.). An academic exchange[19] was organized in December in BiH.

The 2nd CATCHaCORN[20] students' competition was organized by UNSA (22.10.). The 2nd year of experiments was finished. Two master students finished their first year of master studies in Italy (at CIHEAM-IAMB) and one master student finished his master course in BiH (at UNSA). All project reports for 2022 were sent to EC. The dissemination efforts continued - all relevant calls, news and posts were prepared and disseminated to target groups.

Project implementation in 2023 - brief overview

In 2023, SMARTWATER project was presented in Serbia (Zlatibor, Čačak and Belgrade) and Brazil (Fortaleza)[21]. UNSA team organized the 2nd (31.1.-3.2.) and the 3rd (22-24.5.) workshop on EU funding and proposal drafting in Sarajevo.

The 3rd summer school "*Adaptation to climate change of agricultural water management sector in BiH*" was organized by UNI-BL in Trebinje (3-7.7). CIHEAM-IAMB organized the 3rd advanced training course "*Crop growth modelling and water productivity for eco-efficient agricultural water management*" in Valenzano, Bari (17-21.7.).

In 2023, academic exchanges were organized in BiH (Sarajevo[22]) and Portugal (Lisbon) and during main events, like summer schools and training courses. Training materials from these events are being disseminated[23].

During 2023, significant work with project stakeholders was done by the consortium, in terms of the establishment of the research strategy for sustainable agricultural water management in BiH and based on interviews and workshops with stakeholders in different cities in BiH.

The 3rd CATCHaCORN students' competition was organized by UNSA (3.11.). The 3rd year of experiments was finished. Two master students finished their second year of master studies in Italy (at CIHEAM-IAMB).

[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/

[19]https://www.smartwater-project.eu/the-visit-of-professor-todorovic-to-the-university-of-sarajevo-and-banja-luka/

[20] https://www.smartwater-project.eu/harvest-time-ended-this-weekend-with-catchcatctacorn2/

[21] https://www.smartwater-project.eu/news-events/

[22] https://www.smartwater-project.eu/student-exchange-within-the-smartwater-project/

[23] https://www.smartwater-project.eu/training-material/

All project reports for 2023 were sent to EC. SMARTWATER brochure and newsletters for 2023 were prepared and published[24]. The implemented activities within SMARTWATER project in the period 2021-2023 are presented in Table 1.

Table 1. The overview of activities within SMARTWATER project (2021-2023)

no.	*Activity type*	*Accomplished*	*Project report reference*
1	advanced training courses	3/3	D2.2*, D2.4, D2.7
2	summer schools	3/3	D2.1, D2.3, D2.6
3	joint research activities (experiments)	3/3	D3.1, D3.2, D3.3
4	stakeholders' meetings (roundtables)	2/3	D4.1, D4.3
5	post-graduate MSc programs	3/3	D2.8, D2.9, D2.10
6	mutual staff exchanges	10/13	D3.1, D3.2, D3.3
7	hands-on workshops on R&I	3/3	D2.5
8	international conferences	25/3	D3.1, D3.2, D3.3
9	smart water management tools	2/2	D3.1, D3.2, D3.3

** D = deliverable (report)*

Joint experimental studies and publications – basic considerations

Within WP3 experiments at two locations in BiH (Aleksandrovac and Butmir) were performed. The investigated plant species was maize (*Zea mays* L.), hybrid BL 43 (FAO 400 group). The RCB design included two factors, irrigation (3 irrigation levels) and fertilization (2 nitrogen levels). All data were collected and interpreted. Some scientific publications are already available at online repository (Zenodo, 2024) but the consortium is preparing the remaining publications, tackling the main project topics.

The extension of project life - activities in 2024

After the amendment approval by EC, SMARTWATER project life will continue until June 2024. The forthcoming activities include the implementation of remaining academic exchanges, the 3rd stakeholders' meeting, the scientific publishing process and participation in international conferences in order to disseminate SMARTWATER activities.

SMARTWATER team will organize an international workshop in BiH in the period May/June 2024 for all stakeholders in order to present project achievements. We are inviting all interested actors to participate in this event[24].

[24] https://www.smartwater-project.eu/

Conclusions

During the implementation of SMARTWATER project in the period 2021-2023 most pre-planned activities were finished. All project reports for this period are prepared and published. Dissemination was done through different communication channels and is ongoing. In 2024 the consortium will aim to implement the remaining activities in order to fulfill the main project objectives i.e. to upgrade networking and research between project partners and stakeholders and to increase competency and fund raising skills of formed project teams.

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THE USE OF TRACTOR AGGREGATES ON A MEDIUM-SIZED FARM IN ARABLE PRODUCTION

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Abstract: The intensity of use and work output of tractors and implements were investigated using the example of a medium-sized family farm. The farm is located in central Serbia and cultivates 44 ha of arable land for livestock farming. The farm has 3 tractors, 22 attachments, a self-propelled combine harvester for harvesting small grains, and a combine harvester for silage preparation. In the 2021/22 production year, the tractors were used for a total of 376.92 hours, namely 170.58 hours for the FIAT 78 tractor, 144.17 hours for the IMT-539, and 62.17 hours for the IMT-558. Tractors and attachments were most frequently used to produce wheat (10 h ha^{-1}), silage maize (9.94 h ha^{-1}), soybeans (9.72 h ha^{-1}), oats (9.17 h ha^{-1}), barley (8.58 h ha^{-1}) and grain maize (8.17 h ha^{-1}).

The greatest engagement of mechanization was in basic tillage 31%, pre-sowing preparation with a rotary harrow 25%, and the least in spreading mineral fertilizers, protecting plants from diseases, and pests, and destroying weeds 8% of the total working time.

Keywords: family farm, engagement, tractors, attachment machines

Introduction

The high cost of purchasing modern agricultural machinery and the low intensity of use on small plots of land affect their economic profitability. In Serbia, many family farms use tractors, attachments, and harvesting machines that are technically over 30 years old. A two-axle tractor with an average engine power of 32.27 kW (43.52 hp) is used for 421 hours per year to cultivate 6.99 ha of land with 5.89 attachments (Radivojević 2014, Tot and Nikolić 2016, Koprivica et al. 2021, Koprivica et al. 2023).

Rational use of tractors and attachments can be achieved through a greater scope of use, planned sowing, harmonized agricultural techniques, and the

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organization of work on the farm. One of the ways is to join machine groups, rings, and cooperatives, as proposed by Veljković et al. (2020), Tot and Nikolić (2016).

The research aims to show the intensity of the use of mechanization in agricultural production and to calculate the impact of the use of tractors and attachments on a medium-sized family farm. On this basis, a recommendation can be made for the purchase and further equipment with new machines.

Materials and methods

The family agricultural farm "Đurković" is located in the village of Čestin in the municipality of Knić, and in terms of the size of the cultivated land, it belongs to medium-sized farms. It cultivates a total of 44 ha of land, divided into 37 plots with an average size of 1.12 ha. Agricultural production is organized on an area of 14.7 ha in 14 plots with an average size of 1.05 ha. The most common crop is maize on an area of 12 ha, of which 9 ha are used for grain and 3 ha for silage, followed by barley (2 ha), wheat (2 ha), soybeans (1.5 ha) and oats (0.7 ha). Forage crops include fodder turnip on an area of 0.4 ha, alfalfa on 5 ha, and artificial meadows on 20 ha. Crop production is tailored to the needs of livestock farming and the feeding of 18 dairy cows and 13 fattening steers. In terms of sowing structure, 33.41 % or 14.7 ha is devoted to arable crops, 65.67 % or 28.4 ha to forage crops (silage maize is classified as a forage crop), and 0.4 ha or 0.92 % of the total area to vegetable crops.

Based on the measurement of working time by chronography and chronometry, the total operating time of tractors and attachments as well as the consumption of machine working hours per unit area was calculated for each crop in the 2021/22 production year. In addition, the total seasonal use of tractors and attachments for agrotechnical work in the production process of the above-mentioned crops was determined. The work output of tractors with attachments (h/ha) was also calculated. To compare the results obtained, data from literary sources published by foreign and domestic authors were used. The medium size of the average farm corresponds to the classification of Todorović, (2017).

Results and discussion

In the analyzed period 2021/2022, the following agricultural machinery was used in field production on the farm: 3 two-axle tractors IMT 539 (29.5 kW), IMT 558 (42.6 kW), FIAT 78 (57.33 kW), 22 attachments, as well as a small grain combine Zmaj 133 and silage combine Klass Jaguar 70 SF, which are over 40 years old, were used.

On the farm, the average power of the tractor engine is 41.4 kW, and with 7.33 attachments, 14.67 ha are cultivated with an energy output of 2.94 kW/ha according to the studies by Koprivica et al. (2023). In crop production, the working hours were determined according to farm size, sowing structure, number, and category of tractors and implements which are shown in Table 1.

Table 1. Total hours of tractor and attachment use during 2021/2022 production year on the farm

Crops	Surface (ha)	Tractor working hours (h)			Total (h)	Tractor operation (h/ha)	Numb. of plots
		FIAT 78	IMT-539	IMT-558			
Wheat	1.5	10.67	3.83	0.5	15	10	2
Barley	2	12.33	4.5	0.33	17.17	8.58	2
Oats	0.7	4.83	1.17	0.42	6.42	9.17	1
Maize for grain	9	43.67	25.83	4	73.5	8.17	5
Maize for sillage	3	16	13.83	27.5*	29.83	9.94	3
Soybean	1.5	10.17	3.66	0.75	14.58	9.72	1
Totals field crops	17.7	97.67	52.83	6	156.5	8.84	14
Fodder turnip	0.4	11.25	4.67	7	22.92**	57.3**	1
Alfalfa	5	14.17	19.17	11.67	45.10	9.23	6
Meadows	20	47.5	67.5	37.5	152.5	7.62	15
Total	43,1	170.58	144.17	62.17	376.92	8.72	37

**Without time to trample the maize silage of the IMT-558 tractor*
***Time spent mowing, collecting, pressing the green mass of fodder turnip, transporting it to the barn and fresh distributing it every day for feeding cattle.*

In the production of field crops in 2021/22, all three tractors worked a total of 156.5 hours on the Farm. The FIAT 78 tractor was used the most for 97.67 hours, followed by the IMT-539 for 52.83 hours, and the IMT 558 for at least 6 hours. In the 2021/22 season, the total engagement of tractors was 376.92 hours, namely: 170.58 hours FIAT 78, 144.17 hours IMT-539, and 62.17 hours IMT 558 (Table 1).

Research by Škaljić et al. (2017) shows that tractors with 38-66 kW are used for less than 100 h in 23 % of cases, for 100-200 h in 43 % of cases, and more than 200 h in 34 % of cases, which is also confirmed by the individual uses of the tractors in Table 1. The use of FIAT tractors 78 is close to the results of 180 operating hours of a 34.8 kW tractor reported by Stojnović et al. (2001).

In the production of 1.5 ha of wheat, the tractors were used for a total of 15 hours or 10 h ha^{-1}, which is more than the results of Janković (1989) 8.12 h ha^{-1}, Bošnjak et al. (1998) 7.03 h/ha, Obradović and Kresović (1988) 6.56 h/ha, Beck et al. (1988) 8.52 h ha^{-1}, Tot and Nikolić (2016) 8 h ha^{-1}, Koprivica et al (2021) 7.41 h ha^{-1}. The total use of tractors on the Farm in the production of field crops on an area of 17.7 ha is 156.5 h or 8.34 h ha^{-1}, which is in agreement with the results stated by Mago (2009) 8-10 h ha^{-1}, and Tot and Nikolić (2016) 8,7 h ha^{-1}.

The use of tractors and attachment on the Farm by work operations and their chronological order is given in Table 2. Work processes were monitored from their preparation on the economic yard, execution on the plot, and return to the Farm.

Table 2. The work output of tractors and attachment on the Farm by field crop operations in 2021/2022. year

Work operation	Total working time (h)	Work output (h ha^{-1})	Tractor used
Plowing	47.83	2.69	FIAT 78
Soil preparation with rotary harrow	38.67	2.17	
Application of mineral fertilizers	12.42	0.35	IMT-539 Basic fertilization and feeding
Application of pesticides	12.75	0.36	
Sowing	24.83	1.40	
Transportation of grain and silage	8.83	0.74	IMT-539 IMT-558
Pressing, loading, transport, unloading, stacking of roll bales	11.17	0,63	FIAT 78 IMT-558
Total	156.5	8.34	All tractors

In the 2021/2022 production year at the farm, the FIAT 78 tractor was predominantly engaged in basic tillage, specifically using a three-furrow rotary plough for 47.83 hours, accounting for 30.56% of the total operational time. Notably, various studies by Obradović and Kresović (1988), Barać et al. (2013), Kovačev et al. (2013), and Koprivica et al. (2021) indicate that tractors are commonly employed in basic tillage operations. The work output of 2.70 hours per hectare in ploughing, surpassed the findings of Kovačev et al. (2013) but fell short of the results reported by Barać et al. (2013), Obradović and Kresović (1988), and Koprivica et al. (2021). It's important to note that these variations may stem from the distinction between effective work and total work.

In the pre-sowing soil preparation phase, the FIAT 78 tractor utilized a Lemken rotary harrow for 38.67 hours, representing 24.71% of the total operational time. The work output was 2.17 h ha^{-1}, slightly below the reported by Kovačev et al. (2013).

For sowing maize and soybeans, a four-row pneumatic Nodet seeder was employed, while small grains were sown using a mechanical seeder IMT 632. The IMT 539 tractor was engaged for a total of 24.83 hours (15.86%), producing an output of 1.4 h ha^{-1} which is more compared to research by by Obradović and Kresović (1988), Barać et al. (2013), Kovačev et al. (2013), Tot and Nikolić (2016).

The IMT 539 tractor, equipped with the Agrex spreader, operated for 12.42 hours (7.94%) and achieved an output of 0.35 hours per hectare. It's worth noting that this output exceeds the effective work reported by Obradović and Kresović (1988).

The IMT 539 tractor and Crocus sprayer were used for plant protection and weed destruction for 12.75 hours or 8.15% of the total working time. The stated results are lower than the results reported by Koprivica et al. (2021) and higher than the results of Obradović and Kresović (1988).

The results of the research in the paper differ from the results of other authors due to: different technical and operational characteristics of attached machines, tractor power, applied agrotechnics in the production of field crops, distance from the farm, size of the plot, conditions of clima, and type of soil, as well as applied research methods. It can be concluded that the family farm is equipped with agricultural machinery, but the problem is their age and insufficient utilization.

Conclusion

The tractors were used on the farm for a total of 376.92 hours in the 2021/22 production year. The FIAT 78 tractor worked a total of 170.58 hours for the seasonal tasks of plowing, tilling, baling, collecting, unloading, and stacking bales. The IMT-539 (144.17 hours) and IMT-558 (62.17 hours) tractors were used for simpler tasks such as sowing, fertilizer application, transport, hay preparation, and protection against diseases, pests, and weeds. The work output of the tractor is 10 h $ha^{-1}$for wheat production, 9.94 h ha^{-1} for silage maize, 9.72 h ha^{-1} for soybeans, 9.17 h ha^{-1} for oats, 8.58 h ha^{-1} for barley, and 8.17 h ha^{-1} for grain maize.

The analysis of the use of mechanization on the Farm showed that tractors and attachment achieved lower effects than potential due to the size of the property. The increased scope of use is possible by increasing the holding, buying, or leasing land, as well as providing mechanization services to other users.

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LOW-TEMPERATURE STORAGE *IN VITRO* OF FRUIT TREE SPECIES AT THE FRUIT RESEARCH INSTITUTE, ČAČAK

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Abstract: The success of slow-growth storage relies on the interaction of multiple factors. However, despite having a well-designed, species-specific protocol, the response to storage can exhibit notable differences among genotypes, even within the same species. The paper gives a summary of achievements and results in the conservation of several fruit species utilising cold storage technology achieved at the Fruit Research Institute, Čačak. The results of the survival rates of *in vitro* cultures of plums ('Požegača', 'Sitnica', 'Crvena Ranka', Cherry plum), cherry rootstocks ('Gisela 5', 'Tabel Edabriz', 'Colt'), apple ('Gala Must'), and berry fruits ('Meeker', 'Čačanska Bestrna') at +5°C in complete darkness during different periods, as well as their multiplication capacity after cold storage, are presented.

Keywords: cold storage *in vitro*, plum, cherry, apple, berry fruits

Introduction

The medium-term storage of *in vitro* plants using the slow-growth technique is a sophisticated method employed to maintain the genetic integrity and viability of plant cultures over an extended period. It involves manipulating the growth conditions of *in vitro* plants to reduce their metabolic activity, thereby slowing down their growth rate. This is achieved by adjusting the different physical conditions of *in vitro* cultured shoots or the chemical composition of the nutritive medium (Ruta et al., 2020). The technique is generally named slow-growth storage or minimal growth storage, but it may also be called "cold storage" (CS) when low temperatures are applied instead of standard growth conditions. The goal is to create an environment that sustains the plants in a quiescent state, minimising cell division and elongation while maintaining their physiological and genetic stability.

Key considerations for the medium-term storage of *in vitro* plants with slow growth include different aspects. Cold temperatures play a crucial role in inhibiting growth. The temperature range commonly employed for the medium-term preservation of temperate species typically falls between 2°C and 12°C, with 4–5°C being the most commonly utilized range (Lambardi and Ozudogru, 2013). In

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conjunction with the low temperatures, the shoot cultures are usually maintained in complete darkness (Benelli et al., 2022). Nevertheless, some fruit species have shown positive outcomes when subjected to culture maintenance under low light conditions, including variations in photoperiod and reduced light intensity (Kabylbekova et al., 2021; Turdiyev et al., 2020). Providing low light intensity or complete darkness can further suppress metabolic activities and limit the potential for respiration, water loss, drying, and ethylene production (Lambardi and Ozudogru, 2013). Adjustments to the culture medium may encompass alterations to the carbon source content, variations in mineral salt concentrations (both macro and microelements), adjustments to the levels of plant growth regulators in terms of quality and concentration, and the incorporation of growth retardants. Typically, shoot cultures of various plant species are preserved using the same mineral and organic composition of medium employed for proliferation under standard culture conditions. The full-strength Murashige and Skoog medium (MS) is widely used for slow growth storage of temperate fruit species (Sedlak et al., 2019). However, an increase in multiplication eficiency in the *Malus*, *Prunus*, and *Pyrus* genera was observed primarily in ½ MS (both macro and microelements) medium (Arbeloa et al., 2017), as well as in MS medium with reduced nitrogen levels even to ¼ MS (Turdiyev et al., 2020). These effects could be attributed to the nutritive or osmotic impacts of reducing sugar and mineral nutrients. Regarding the types and concentrations of both carbon sources and plant growth regulators (PGR), they affected the slow growth storage of fruit tree species, but this effect is highly genotype-specific (Sedlak et al., 2019; Arbeloa et al., 2017; Kabylbekova et al., 2021). In addition to utilising temperature and osmotic agents for short- to medium-term storage in germplasm conservation, growth retardants like abscisic acid. They are known for varied physiological effects on plants, including the inhibition of plant elongation and cell division and the accumulation of stress-resistant proteins (Benelli et al., 2022); however, some of them display mutagenic properties that can lead to mutations and physiological issues with prolonged use.

Given the importance of slow-growth storage for maintaining the viability and accessibility of *in vitro* collections, the Tissue Culture Laboratory at the Fruit Research Institute (FRI) in Čačak has, since its establishment, effectively employed the slow growth technique at +5°C in complete darkness. This method has been utilized to establish *in vitro* collections of various temperate fruit tree species, aimed at short- and medium-term preservation, facilitating exchange, and enabling rapid propagation as needed. The paper provides an overview of achievements and results in the conservation of various stone, pome, and berry fruit species using the CS technique obtained at FRI in Čačak.

Low-temperature storage *in vitro* of fruit germplasm at FRI

Considering the significance of indigenous plum cultivars to Serbian tradition, customs, legacy, and cultural identity, and recognising that many of these cultivars are currently facing serious threats and gradually disappearing from orchards, the primary focus of the FRI has been to explore the potential for cold storage of *in vitro* plum shoots, particularly emphasising autochthonous genotypes in the previous period (Vujović et al., 2022). Shoot cultures of plum 'Požegača' were stored successfully at 5 °C for 10 months with 100% survival and no signs of necrosis or dessication observed (Ružić and Cerović, 1990). The assessment of 'Požegača' shoot multiplication capacity during CS revealed a notably high multiplication index at 6 and 10 months of CS (Vujović et al., 2023). The extent of multiplication varied based on the duration of shoot growth under standard growth conditions (SC) prior to CS, with the highest multiplication index achieved in shoots cultivated for 21 days under SGC followed by six months of CS. According to Ružić and Cerović (1990), a subcultivation period of 21 days was found to be the most favorable before subjecting *in vitro* shoots to CS. In contrast to 'Požegača', shoots of the plum genotype 'Sitnica' exhibited heightened sensitivity to CS conditions, resulting in 44% and 82.9% of shoots undergoing complete necrosis after 6 and 9 months of exposure, respectively (Ružić et al., 2012). Among the plantlets that managed to survive the storage period, they demonstrated partial viability, characterised by necrotic axial shoots and chlorotic laterals. Notably, under identical CS conditions, shoots of the Cherry plum manifested even greater sensitivity, with a survival rate of only 12.5% observed after 6 months, and no viable shoots remaining after 9 months of storage (Ružić et al., 2015a). For both genotypes, the highest viability and regrowth were observed after 3 months in CS. Shoots were viable and etiolated, although sporadic instances of necrosis were observed on leaves in the basal section. The transition of cultures to SGC resulted in the rapid development and greening of leaves, accompanied by the restoration of morphology and the ability for multiplication. Vujović et al. (2020) also investigated the capacity for slow-growth storage of the plum 'Crvena Ranka' through temperature reduction combined with incubation in complete darkness. The results revealed that *in vitro* shoots could be conserved over the medium term under CS conditions for up to six months. High survival rates were achieved after three months (94%) and six months (82.5%), while severe signs of necrosis (100%) were observed after nine months of preservation. Shoots subcultured under SGC after CS promptly regained their morphology, although their capacity for multiplication and rooting was slightly lower than that of non-cold-stored shoots.

Research investigating the preservation capacity of cherry shoots under CS conditions began in the 1990s at FRI's Laboratory for Tissue Culture, with a primary focus on cherry rootstocks. The study discovered that both 'Gisela 5' and 'Colt' cherry rootstocks had good survival rates following three months of CS, with 'Gisela 5' outperforming 'Colt'. CS significantly impacted shoot multiplication and growth performance, with 'Gisela 5' exceeding 'Colt' in terms of multiplication index, shoot length, and leaf count (Ružić and Cerović, 1999). Further research on 'Gisela 5' revealed that it was possible to successfully store *in vitro* shoots of this genotype for up to six months in CS conditions with a high survival rate of 95%. However, after nine months, a significant fall in survival (45%) was found (Ružić et al., 2015b). In both cases, cold-stored plants had necrotic axial shoots and live laterals. Shoots regained normal morphology already in the first subculture following CS, similar to non-cold-stored shoots; however, they did not regain multiplication capacity, as measured by the multiplication index and length of axial and lateral shoots, even by the third subculture. Vujović et al. (2023) also conducted a study on 'Gisela 5' and 'Tabel Edabtiz' shoots following a three-month CS period. The results indicated a significant increase (1.7 to 4.8 times) in the multiplication index of shoots compared to those grown under SGC for both rootstocks. Additionally, 'Gisela 5' showed a superior multiplication index and longer shoots after 21 days in CS compared to 'Tabel Edabriz.' Despite the observed etiolation and sporadic necrosis in the base section, the survival rate was 100%, and upon transferring to SGC, the shoots rapidly recovered their morphology, regained their capacity for multiplication, and exhibited leaf greening.

In vitro storage of the apple ‘Gala Must’ under CS conditions for 9 months revealed five types of shoots (Ružić et al., 2016): i) fully viable shoots, ii) viable shoots with leaf necrosis, iii) etiolated shoots with leaf necrosis, iv) necrotic axial shoots with etiolated laterals, and v) fully necrotic shoots. The apple culture showed a remarkable level of resilience to CS conditions, with fully necrotic shoots appearing in a small percentage (10%) after 9 months of CS. Fully viable shoots (non-etiolated with no evidence of necrosis on the stem or leaves) were observed only after three months of CS, whereas all other shoots were viable but had leaf necrosis. As shoot storage duration increased, the percentage of etiolated shoots with leaf necrosis increased (88.89% after 6 months), as did the percentage of necrotic axial shoots with etiolated laterals (90% after 9 months). When cultures were transferred from the cold chamber to SGC, the leaves in the shoots recovered promptly and turned green, regaining their morphology and capacity for multiplication and rooting. The recorded multiplication index improved towards the third subculture, and the shoots rooted effectively at a rate ranging from 66.8% to 80%.

The shoots of the raspberry ‘Meeker’ were successfully maintained in CS, with a high survival rate exceeding 80% (Ružić et al., 2009). The multiplication index and shoot length were higher in the first subculture after CS than in shoots cultivated under SGC. The pH value of the growth medium increased during and after CS, and slow growth was accompanied by a gradual uptake of nitrogen from the medium. These findings indicate that it is possible to improve raspberry multiplication and shoot length by replacing the natural dormancy phase with short-term maintenance in CS. The minimal nitrogen uptake from the medium suggests that nitrogen addition is unnecessary while storing raspberry 'Meeker' under CS conditions. Indeed, a significant amount of this element remained in the medium even after three months of CS, and plant growth and development were unaffected. Contrary to previous results, a very low percentage of encapsulated shoot tips of this genotype (6.3%) and blackberry Čačanska Bestrna (18.8%) survived the one-month CS period and exhibited regrowth (Ružić et al., 2011).

Conclusion

The results presented highlight the significant variations in storage duration among different fruit genera and across genotypes. Consequently, it is imperative that efforts not only concentrate on formulating genotype-specific protocols for micropropagation but also encompass slow-growth storage considerations.

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THE COMPARATIVE ANALYSIS OF EUROPEAN WINE INDUSTRY

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Abstract: This document presents a comprehensive overview of European wine industry, covering changes in production, consumption and the market. We have rebuilt the data collection from online sources and statistical information of International Organization of Vine and Wine (OIV), which are available through Statista. Currently, there are a large number of methods of growing and producing wine in the wine industry in Europe. The European wine market also includes its production, which is related to the area of vineyards and its consumption. Among the largest consumers of wine according to population in the world is Europe, with traditional countries such as Italy, France, Spain and others.

Keywords: wine industry, wine production, wine consumption, economic significance.

Introduction

The wine production belongs into the global market dealing with wine which is directly connected to the acreage of vineyards and its consumption. Today`s market with wine is spread all over the world. There is a huge number of wine producers and consumers in Europe. Nowadays, there are many ways of cultivating and production of wine in wine growing industry in Europe. We can meet the traditional small wine producers who still keep processing wine manually as well as big machine- worked industrial producers of wine can be met. Wine industry in the world as well as in Europe is a subject to wine acts that are determined by regulations for particular qualitative levels of wines that can differ in content and strictness of a particular regulation in each state. According to the production, wine world can be divided into following areas: Europe, New World where Australia, North and South America, South Africa belong and the newest world (China, India, Brazil, North Africa, and Eastern

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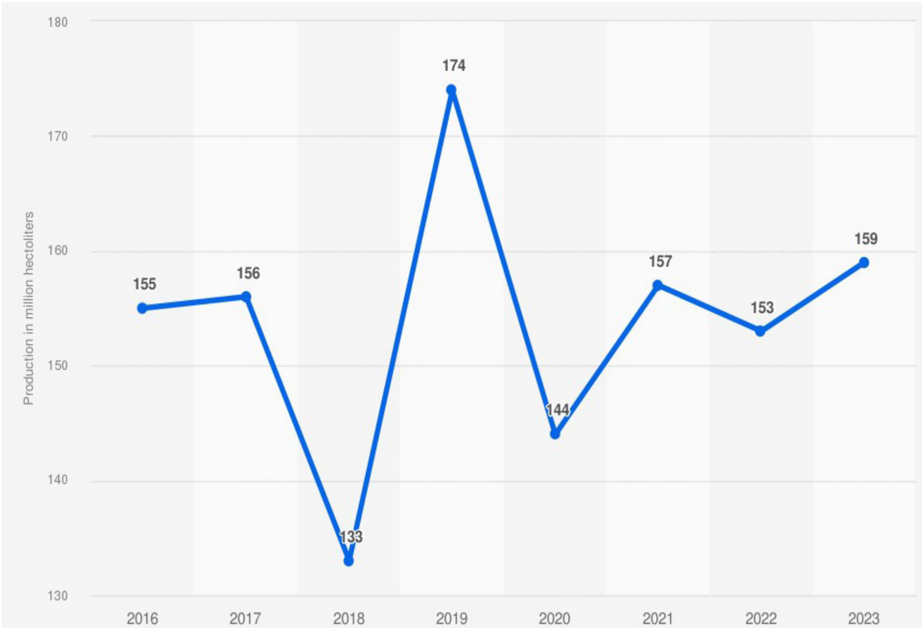
Europe). These countries, with their availability of resources and economies of scale, have gained market share and disrupted the traditional dominance of established wine-producing nations (Morrison & Rabellotti, 2017; Cusmano et al., 2010).

Materials and methods

The research is based on the review of scientific and professional literature and uses general scientific methods. We particularly emphasize the specific methods and tools as statistical and economic indicators and the composite EU production, sale and consumption of wine. To gather comprehensive data on EU wine production, our methodology involved a combination of data mining from internet sources and the use of statistical information provided by Statista. In our study, we used the dataset source which is accessible through the renowned online platform, Statista. Statista provides access to a vast array of EU statistics and market data.

Results and discussion

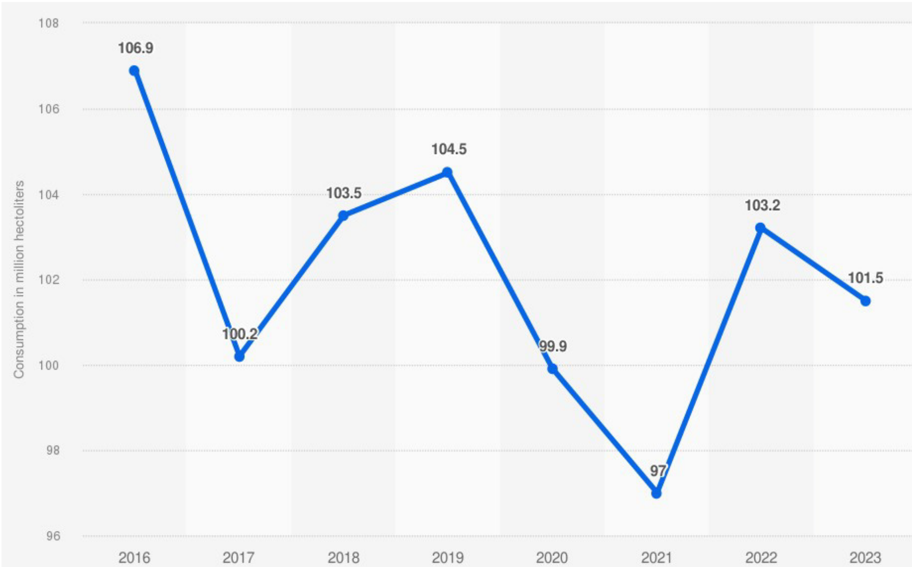
The world's largest wine production in Europe is Italy, France and Spain together produces almost 50% of the world's production. The economic aspect of wine production is remarkable and therefore it is the main reason why governments support strong research programs in the field of development and improvement of wine industry (Bisson a kol. 2002). Many traditional European countries that produce and sell wine are active in the international wine trade.



Graph 1. Wine production in the European Union from 2016 to 2023 (in million hectoliters)

Wine production in the European Union grew to 159 million hectoliters in 2022. This was a rebound from the 2.55 percent drop in 2022 (Statista 2024).

Wine in Europe as well as all around the world is a very popular trading commodity while wine industry and the trade with wine as such is subject to many regulations. Wineries must now navigate this global market and align their strategies with market challenges while emphasizing environmental responsibilities (Faria et al., 2021). Wine industry creates the significant part of economy in many European countries and it generates strong production and consumption.

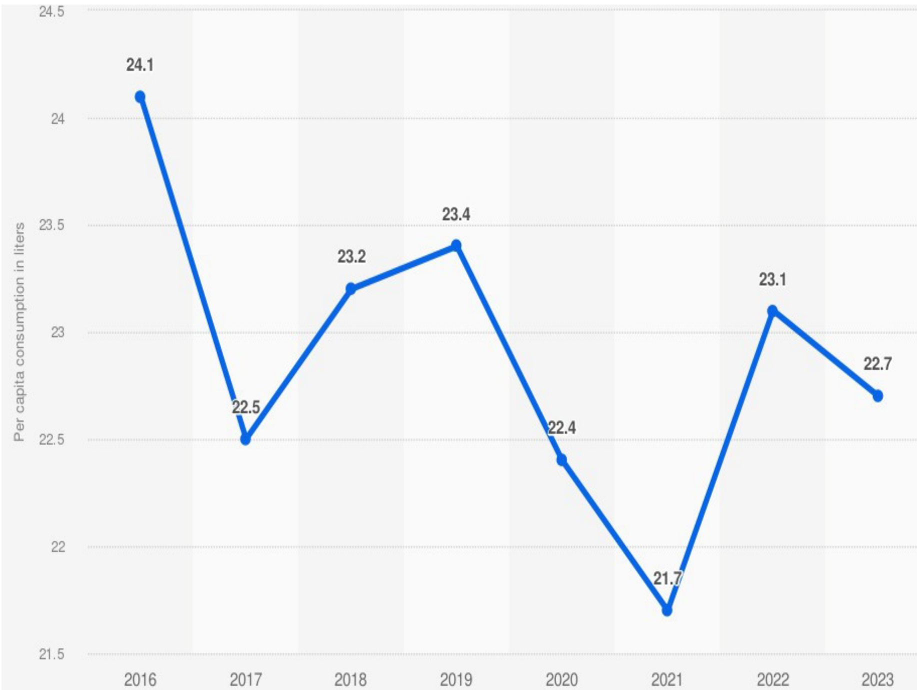


Graph 2. Annual wine consumption in the European Union from 2016 to 2023 (in million hectoliters)*

Wine consumption in the European Union declined to 101.5 million hectoliters in 2023. This represented a further decline from the 2016 peak of 107 million hectoliters although still up from the pandemic low of 97 million hectoliters (Statista 2024).

Per capita wine consumption in the European Union

The production of wine belongs into the European market with wine which is connected to the acreage of vineyards and its consumption what significantly influences international flows. Europe with traditional countries like Italy, France, Spain and others belong into the biggest consumers of wine according to the number of inhabitants.

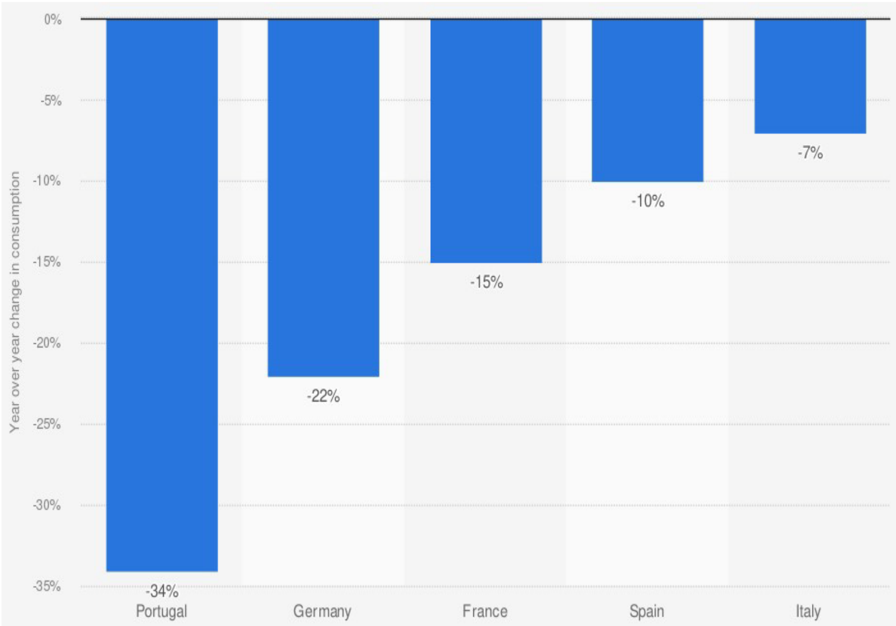


Graph 3. Per capita wine consumption in the European Union from 2016 to 2023 *(in liters)*

Per capita wine consumption in the European Union declined to 22.7 liters in 2023. This was an additional reduction from the 2016 peak of 24.1 liters but up from the pandemic low of 21.7 liters (Statista 2024).

Change in the consumption of wine in the European Union

Globalization has paved the way for significant changes in the wine industry worldwide. We can state that the biggest changes in world wine production happened in the last 10 years and were caused by its changes and development. This threatened the industry's dominance by the traditional countries (Cusmano et al. 2010), who faced rising challenges due to increased competition, changes in consumer behaviour, and growing environmental and public health concerns. There was also a decrease in wine production in Europe.



Graph 4. Change in the consumption of wine in select countries in the European Union in 2023

Wine consumption declined by 34 percent in Portugal in 2023 compared to the previous year. This was the greatest reduction in consumption among the largest wine markets with the European Union (Statista 2024).

Conclusion

European wine industry creates the significant part of economy in many countries and it brings faster growth of the volume of production and the export value. European wine market as well as other markets is led by market power. In Europe the significant focus has to be put in the future mainly on the development in the field of viniculture accompanied by many innovations and modern inventions. The Internet creates new possibilities in how to impress directly customer market segments with new market participants and new screenplays from the point of view of production and consumption.

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APPLICATION OF MULTIPLEX RT-PCR FOR GRAPEVINE VIRUSES DETECTION

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Abstract: Grapevine, a significant fruit crop globally, is a host of various viruses that negatively affect yield, plant vigor, and fruit quality. Multiplex polymerase chain reaction (mPCR) offers the ability to detect numerous viruses simultaneously. Our study aimed to evaluate the effectiveness of mRT-PCR for the detection of nine grapevine viruses in Serbia, including: grapevine fanleaf virus, grapevine leafroll-associated viruses 1, 2, and 3, grapevine rupestris stem pitting associated virus, grapevine virus A, grapevine virus B, grapevine fleck virus, and arabis mosaic virus. This study confirms mRT-PCR as an efficient method for simultaneous detection of multiple grapevine viruses.

Keywords: grapevine, viruses, detection, mRT-PCR

Introduction

Grapevine (*Vitis vinifera* L.) is considered one of the most important fruit crops worldwide. Due to its high impact on the socioeconomy, great attention is focused on the cultivation and protection of this culture. Nevertheless, a grapevine is known to host a large number of different viruses that reduce yield and plant vigor, decrease fruit quality, and shorten vine longevity (Djenane et al., 2021). So far, more than 80 viruses have been discovered to infect grapevines. Leaf degeneration, grapevine leafroll, rugose wood complex, and fleck disease are the four main disorders among viruses of worldwide economic importance (Basso et al., 2017; Martelli, 2017; Vu et al., 2023).

Grapevine fanleaf virus (GFLV) is the main causative agent of grapevine fanleaf disease. GFLV causes many symptoms including leaf deformation, yellowing, mosaicking, vein banding, abnormal branching, and shortened internodes. Also, it causes considerable crop losses, reduces fruit quality, and shortens the longevity of grapevines in the vineyard. Yield losses can reach 77% in the most severe cases. It is

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transmitted specifically by the ectoparasitic nematode *Xiphinema index*, and belongs to the genus *Nepovirus* (Andret-Link et al., 2004; Djenane et al., 2021).

A group of viruses known as grapevine leafroll-associated viruses (GLRaVs), with at least 12 serologically distinct variants identified, named GLRaV-1 to GLRaV-12 are associated with leafroll disease. Ten of these viruses belong to the *Ampelovirus* genus, GLRaV-2 and GLRaV-7 belong to the *Closterovirus* and *Velarivirus* genus, respectively. The most widespread leafroll viruses are GLRaV-1 and GLRaV-3, followed by GLRaV-2 (Gambino, 2015; Martelli, 2017). Symptomatic vines infected with GLRaV-3 showed less vigorous growth and lower fruit yields compared to healthy ones. These viruses are primarily spread through vegetative propagation and grafting. The transportation of planting and propagation materials facilitates the long-distance movement of GLRaVs (Alabi et al., 2016).

Grapevine rupestris stem pitting associated virus (GRSPaV) is a member of the genus *Foveavirus* and is linked with Rupestris stem pitting disease. A close association between the presence of GRSPaV and symptoms of vein necrosis has been recently observed (Gambino and Gribaudo, 2006). GRSPaV is perhaps the most prevalent grapevine virus, and it can occur as distinct variants, each showing different symptoms on various *Vitis* spp (Meng and Rowhani, 2017). The presence of GRSPaV together with other viruses causes severe damage to infected grapevine plants very often. So, it's indicated that the presence grapevine virus A (GVA), may be required for rugose wood symptoms to occur. The virus is grapevine-specific and spreads through vegetative propagation, grafting, and possibly pollen and seed. However, no biological vectors have been reported (Stankovic et al., 2023).

The most widespread virus within the fleck complex is grapevine fleck virus (GFkV), which is the type species of the genus *Maculavirus*. Although latent in *V. vinifera*, GFkV induces specific foliar symptoms in the indicator *V. rupestris*, appearing as translucent spots. Leaves with intense flecking become wrinkled, twisted, and may curl upward. In contrast to the other viruses reported only in specific geographical areas, GFkV is ubiquitous. All these viruses are primarily spread through infected propagating material (Martelli, 2017).

Multiplex polymerase chain reaction (mPCR) is the simultaneous detection of multiple targets (e.g. viruses) in a single reaction using different primer pairs of each target. This method, which expands on the practical use of PCR, can result in significant time and labor savings in the laboratory without compromising the usefulness of the experiment. Multiplex PCR has been developed for the detection of multiple viruses in different plant species, including grapevine, cucurbits, wheat, potato, and blueberry (Gambino and Gribaudo, 2006; Kwon et al., 2014; Kumar et al., 2017; Lee et al., 2023; Deb et al., 2023).

Multiple infections in grapevines are frequent, and the use of one reaction for the detection of several viruses is of great importance to facilitate the analysis. The aim of our study was to evaluate the multiplex reverse transcription-polymerase chain reaction (mRT-PCR) for simultaneous detection of nine viruses infecting grapevines in Serbia.

Materials and methods

The material for this study was selected from the collection of grapevine plants maintained at the greenhouse of the Fruit Research Institute, Čačak. The collection was formed in 2020 by rooting hardwood cuttings collected in grape orchards in Rasina district, Serbia. Cuttings were collected from plants showing various symptoms on leaves that could be ascribed to viral infections and from asymptomatic plants. Leaf samples for the analysis were collected from two-year-old plants and stored at -20°C until analysis. For this study, a total of 13 samples were selected and analyzed (Table 1).

Table 1. The list of tested samples used in this study

Sample number	Cultivar	Locality
1	Smederevka	Trmčare
2	Prokupac	Trmčare
3	Prokupac	Kobilje
4	Prokupac	Kobilje
5	Prokupac	Trmčare
6	Rajnski Rizling	Trmčare
7	Tamjanika	Trmčare
8	Smederevka	Trmčare
9	Prokupac	Trmčare
10	Prokupac	Trmčare
11	Prokupac	Mala Kruševica
12	Grakče	Padež
13	Prokupac	Donja Zleginja

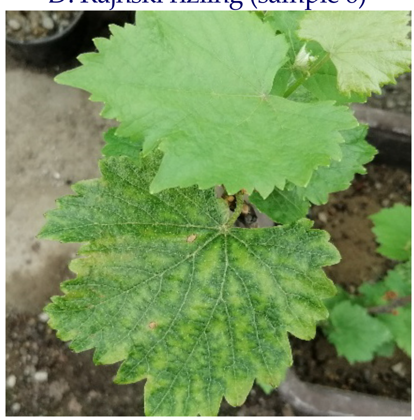
A. Smederevka (sample 1)

B. Prokupac (sample 2)

C. Prokupac (sample 3)

D. Rajnski rizling (sample 6)

E. Prokupac (sample 11)



F. Prokupac (sample 13)

Figures 1A–1F. Symptoms associated with virus infections on grapevine cultivars

Total nucleic acids (TNA) were extracted from frozen leaf petioles using a 2% CTAB buffer, following the protocol of Li et al. (2008) with minor modifications as described by Jevremović et al (2019). The first-strand cDNAs were synthesized by reverse transcription (RT) reactions using Maxima Reverse Transcriptase (Thermo Fisher Scientific, USA) in accordance with the manufacturer's protocol. The obtained cDNAs were used as templates in multiplex polymerase chain reaction with nine virus-specific primer pairs (Table 2). The mPCR conditions were as follows: initial denaturation at 94°C for 4 min; followed by 35 cycles at 94°C for 30 s, 50°C for 1 min and 72°C for 90 s; with a final extension at 72°C for 10 min. mRT-PCR reactions were conducted in TPersonal thermocycler (Biometra, Germany) and PCR products were analyzed on 5% polyacrylamide gel stained with silver-nitrate.

Table 2. Specification of primers used for virus detection

Virus	Primers	Primes sequences 5'-3'	Product size (bp)	Reference
GFLV	Forward Reverse	ATGCTGGATATCGTGACCCTGT GAAGGTATGCCTGCTTCAGTGG	118	Gambino and Gribaudo (2006)
GRSPaV	Forward Reverse	GGGTGGGATGTAGTAACTTTTGA GCAAGTGAAATGAAAGCATCACT	155	
GFkV	Forward Reverse	TGACCAGCCTGCTGTCTCTA TGGACAGGGAGGTGTAGGAG	179	
GLRaV-1	Forward Reverse	TCTTTACCAACCCCGAGATGAA GTGTCTGGTGACGTGCTAAACG	232	
GVA	Forward Reverse	GAGGTAGATATAGTAGGACCTA TCGAACATAACCTGTGGCTC	272	
GLRaV-3	Forward Reverse	TACGTTAAGGACGGGACACAGG TGCGGCATTAATCTTCATTG	336	
ArMV	Forward Reverse	TGACAACATGGTATGAAGCACA TATAGGGCCTTTCATCACGAAT	402	
GVB	Forward Reverse	GTGCTAAGAACGTCTTCACAGC ATCAGCAAACACGCTTGAACCG	460	
GLRaV-2	Forward Reverse	GGTGATAACCGACGCCTCTA CCTAGCTGACGCAGATTGCT	543	

Results and discussion

PCR analysis revealed virus presence in all 13 tested samples (Table 3). PCR products of the expected size were obtained from tested grapevines, and no amplification products were observed in the negative control (sterile water). Positive reactions were obtained with positive controls of tested viruses (Figure not shown). Seven out of nine tested viruses were detected in analyzed samples: GFLV, GRSPaV, GFkV, GLRaV1, GLRaV3, ArMV and GVA. The most detected virus was GRSPaV that was confirmed in all 13 samples, followed by GLRaV-1 (12), GFLV (9), GLRaV-3 (8), GFkV (4), GVB (2), and ArMV (1). The presence of GVA and GlRaV-2 was not confirmed in tested samples.

Table 3. Results of mRT-PCR detection in grapevine samples

No	**virus**								
	GFLV	GRSPaV	GFkV	GLRaV1	GVA	GLRaV3	ArMV	GVB	GLRaV2
1	+*	+	+	+	-**	+	-	+	-
2	-	+	-	-	-	-	-	-	-
3	-	+	-	+	-	-	-	-	-
4	-	+	-	+	-	+	-	-	-
5	+	+	+	+	-	+	-	-	-
6	+	+	-	+	-	+	+	+	-
7	-	+	-	+	-	-	-	-	-
8	+	+	-	+	-	-	-	-	-
9	+	+	-	+	-	+	-	-	-
10	+	+	-	+	-	+	-	-	-
11	+	+	+	+	-	+	-	-	-
12	+	+	-	+	-	-	-	-	-
13	+	+	+	+	-	+	-	-	-

*Positive reaction, **Negative reaction

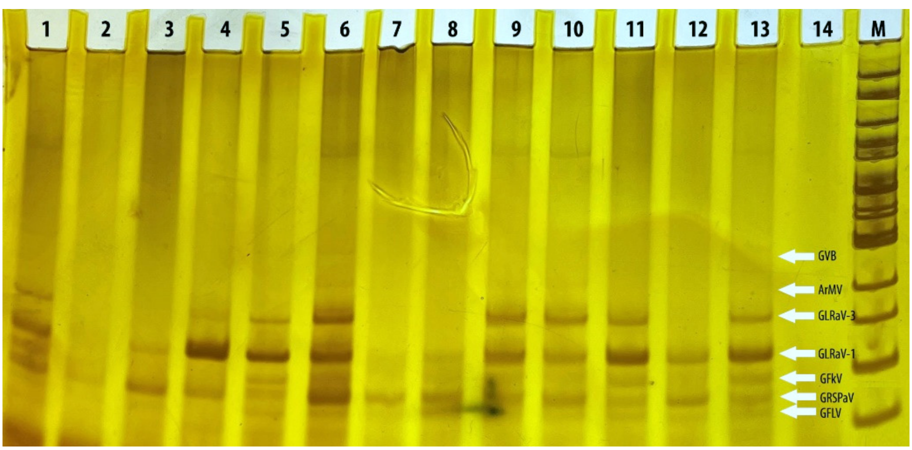


Figure 2. mRT-PCR detection of grapevine viruses (lines 1–13: analysed samples, line 14: negative control; line M: 100 bp DNA Ladder (Solis BioDyne, Estonia)

Lanes 1–13 (Table 2) show bands of different intensities for the desired PCR products around the expected size. In general, stronger bands indicate relatively higher virus concentration in tested sample. As presented in figure 2, in all tested samples, except sample 2, mixed infections were detected.

Several research groups reported simultaneous detection of grapevine viruses. Nassuth et al. (2000) published mRT-PCR procedure for the detection of five grapevine viruses: GVA, GVB, GLRaV-3, ArMV and GRSPaV. Gambino and Gribaudo (2006) used a multiplex procedure to detect the nine most important and widespread viruses of grapevine. A large number of samples were tested using ELISA, single-PCR and mRT-PCR. Based on the obtained results, the authors recommended the use of sensitive and reliable mRT-PCR for analysis in order to reduce costs and save time. López-Fabuel et al. (2013) developed a real-time RT-PCR multiplex detection method for the detection of five major grapevine viruses (GFLV, ArMV, GLRaV-1, GLRaV-3, and GFkV). Later on, the authors upgraded the method, including four viruses: GVA, GVB, GRSPaV, and GLRaV-2. This approach improved detection by broadening the range of targeted viruses. Bruisson et al. (2017) successfully identified the same set of nine grapevine viruses by TaqMan® RT-qPCR.

In previous research, Gambino (2015) demonstrated that the detection limits of multiplex RT-PCR were lower than those of single RT-PCR (primer sets undergoing separate testing in sRT-PCR before multiplex analysis). The reason is that, in the multiplex assay, the mix of primers competes for all templates instead of just one. Furthermore, compared to other advanced methods like real-time RT-PCR, the multiplex protocol did not show greater sensitivity. However, the level of sensitivity is considered reliable enough for the proposed purpose. It offers an effective and quick diagnostic process that's reliable, affordable, and easy to use, even for labs without much expertise or expensive equipment. In our study, the performance of mRT-PCR was reliable for routine diagnostics, allowing quick and multiple detection of viruses infecting grapevines. The method is recommended for rapid screening of the material. For certification purposes, to produce nuclear stock and to analyze higher categories of planting material, a combination of different methods is recommended (biological, serological, and molecular).

So far, eleven viruses infecting grapevines have been identified in Serbia: GVA, GVB, GLRaV-1, -2, -3, GFkV, ArMV, GFLV, raspberry bushy dwarf virus (RBDV), GPGV (grapevine Pinot gris virus), and GRSPaV (Sivčev et al., 2011; Jevremović and Paunović 2011; Mandić, 2018; Živković et al., 2022; Stanković et al., 2023). In all these studies, serological (ELISA) and molecular RT-PCR analyses were applied. In our study we presented the results of the application of mRT-PCR for the simultaneous amplification of several RNA plant viruses. This technique developed by Gambino and Gribaudo (2006) was found to be effective for detecting Serbian isolates of viruses infecting grapevines. Based on the results, this protocol can be recommended for the analysis of grapevine in Serbia, which allows rapid and simultaneous detection of multiple viruses.

Conclusion

The findings of our investigation confirmed the efficiency and suitability of multiplex RT-PCR for the simultaneous detection of multiple viruses in grapevine samples. The method was successfully applied for the detection of Serbian isolates of grapevine viruses, both in single and multiple infections.

Acknowledgement

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PHENOLOGY AND FRUIT CHARACTERISTICS OF AUTOCHTHONOUS APPLE CULTIVARS

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Abstract: In 2014, we evaluated the main phenological and fruit physical-chemical characteristics of apple cultivars grown in western Serbia. The ‘Idared’ cultivar had the earliest start of flowering on March 30, immediately followed by the ‘Kožara’ cultivar on March 31. The cultivars ‘Kiseljaja’, ‘Budimka’ and ‘Kolačara’ cultivars began flowering at the same time on April 3. The ‘Idared’ cultivar was the first to reach full bloom on April 4, followed by the ‘Kožara’ cultivar on April 5. The other cultivars reached full flowering 3 to 4 days after the control cultivar. The results showed significant differences between cultivars in all fruit sizes, fruit weight (g), flesh firmness (kg cm^{-2}), TSS (°Brix), TA (%) and RI.

Key words: flowering, leaf, apple fruit characteristics.

Introduction

Serbia is a country with a very long tradition of fruit growing, in which autochthonous apple cultivars play a very important role. Their share and importance were very high, in the past. However, old autochthonous cultivars have disappeared throughout the Balkans because not enough attention is paid to them (Vujević, 2016).

In the Čačak area, various autochthonous apple cultivars are grown both in gardens and orchards. Their importance is reflected in the fact that they are very suitable for organic cultivation. The indigenous cultivars have genes responsible for certain traits such as resistance to pathogens and pests, color, aroma, resistance to abiotic environmental factors (Skendrović Babojević et al., 2014), suitability for processing into juices, fruit wine, fruit vinegar, brandy and for drying (Milenković and Lukić, 2008).

The aim of this study is to investigate the most important characteristics of autochthonous apple cultivars in the Čačak area, with a special focus on phenological and pomological characteristics.

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Materials and methods

The experiment was conducted in 2014 in an apple orchard in the village of Gornja Gorevnica, 9 km northwest of Čačak (20°57'48″ N; 20°19'31″ E; 396 m above sea level). In the orchard, the usual care measures carried out in intensive apple orchards were applied, with no irrigation.

The study included four autochthonous apple cultivars (‘Kožara’, ‘Kolačara’, ‘Budimka’ and ‘Kiseljaja’), which were compared with the ‘Idared’ cultivar, the most commonly cultivated cultivar in Serbia (control). The cultivars were grafted onto a rootstock M.9 and planted in 2010 with a planting distance of 4 × 1 m, which corresponds to a planting density of 2,500 trees ha^{-1}.

The dimensions of the leaf (mm) and the length of the petiole (mm) were measured with a ruler. The fruit dimensions were determined with an INOX brand 1/20 mm caliper. The fruit weight (g) was measured with a KERN FCB technical balance (Kern & Sohn GmbH, Belingen, Germany), measuring range 6100 g, with an accuracy of ±0.2 g. Flesh firmness (kg cm^{-2}) was measured with a Bertuzzi FT-327 manual penetrometer (Facchini, Alfonsine, Italy). Arithmetic fruit diameter (mm), geometric fruit diameter (mm), sphericity and fruit surface area (cm^2) were calculated according to the formulas proposed by Mohsenin (1986). Fruit elongation (Ra) was calculated as the ratio between length and width (L/W ratio). The content of TSS (°Brix) in the fruit was determined using a Milwaukee MR 200 handheld refractometer (ATC, Rocky Mount, USA) and the content of total acids (%) was determined by titration with 0.1N NaOH. The ripening index (RI) of the fruit was determined as a quotient between the content of TSS and TA.

Differences between data were determined separately by two-way analysis of variance (ANOVA) using the GenStat software package (VSN International, Harpenden, UK). Mean values were compared using the LSD test at $P \leq 0.05$

Results and discussion

The results of the work with regard to the start, full flowering and end of flowering are shown in Table 1.

The ‘Idared’ cultivar had the earliest onset of flowering and the earliest full flowering, immediately followed by the ‘Kožara’ cultivar. The cultivars ‘Kiseljaja’, ‘Budimka’ and ‘Kolačara’ started flowering at the same time. The approximate duration of the flowering period was between 15 and 17 days for all cultivars.

The flowering phase of the apple is the most important and critical phase in the annual cycle as yield often decreases or does not occur at all due to the occurrence of late frosts in spring (Milošević, 1997). Beber (2009) also agrees, stating that knowledge of the flowering time of apple cultivars is of great importance for successful production. This is due to the frequent occurrence of spring frosts, which can damage up to 70% of the blossoms on the tree.

Table 1. Flowering characteristics of defferent apple cultivars

Cultivar	Flowering			
	The begining	Full flowering	The end	Duration
'Kožara'	March 31	April 05	April 15	16
'Kolačara'	April 03	April 09	April 20	17
'Budimka'	April 03	April 08	April 19	16
'Kiseljaja'	April 03	April 08	April 18	15
'Idared'	March 30	April 04	April 15	17

Under our conditions, when the years are with usual climatic factors, the apple blossoms from April 15 to May 10, according to Milošević (1997). However, it can be seen from Table 1 that the cultivars started flowering much earlier, which may be a consequence of the climatic conditions in the year of the study. On the other hand, our results are in agreement with the results of Božović et al. (2015).

The results related to the dimensions of the leaf and its petiole are presented in Table 2.

Table 2. Leaf characteristics of different apple cultivars

	Leaf dimensions			Leaf petiole
	Length (mm)	Width (mm)	C^1 (mm)	Length (mm)
'Kožara'	79.45±2.11 b	64.65±2.07 a	30.65±1.17 b	26.78±1.17 b
'Kolačara'	76.70±2.43 b	60.01±1.88 a	29.60±1.08 b	28.15±1.09 b
'Budimka'	73.90±3.07 b	47.00±1.35 b	35.60±1.44 b	29.58±1.90 b
'Kiseljaja'	92.60±3.32 a	64.40±2.39 a	46.38±1.98 a	34.80±2.03 b
'Idared'	100.50±3.50 a	57.18±2.50 a	44.60±1.56 a	43.03±2.05 a
ANOVA	*	*	*	*

Different lowercase letters in a column indicate significant differences between means for $P \leq 0.05$ by LSD test; C^1 distance of the maximum width of the leaf from the base of the leaf plate

In terms of the leaf length, the cultivars 'Idared' and 'Kiseljaja' had the highest values. In contrast, cultivars 'Kožara', 'Kolačara' and 'Budimka' had a significantly shorter leaf length than the control cultivar. The cultivars 'Kožara', 'Kiseljaja', 'Kolačara' and 'Idared' had the greatest width, although the differences between them were not significant. ‘Budimka’ had a significantly smaller leaf width than the other cultivars. ‘Kiseljaja’ and ‘Idared’ had the greatest distance of the largest leaf width from the base of the leaf surface. The longest petiole was found in the ‘Idared’ cultivar.

High values of leaf dimensions in ‘Idared’ cultivars were also reported by Živaljević (1986).

Table 3. Phisical characteristics of fruit and fruit petiole

Cultivar	Fruit length (mm)	Fruit width (mm)	Fruit weight (g)	Length of fruit petiole (mm)	Flesh firmness (kg cm^{-2})
‘Kožara’	81.95 ± 3.17 a	95.05 ± 3.71 a	347.90 ± 14.20 a	16.40 ± 0.78 a	4.90 ± 0.17 a
‘Kolačara’	61.82 ± 2.27 b	92.40 ± 3.56 a	231.60 ± 12.29 b	4.10 ± 0.23 c	4.26 ± 0.23 b
‘Budimka’	63.70 ± 2.50 b	87.47 ± 3.02 ab	247.00 ± 10.56 b	12.77 ± 0.65 b	4.42 ± 0.25 b
‘Kiseljaja’	73.47 ± 3.38 ab	79.57 ± 2.90 b	200.30 ± 9.09 c	9.30 ± 0.45 b	3.85 ± 0.12 c
‘Idared’	68.02 ± 3.14 b	82.50 ± 3.44 b	225.07 ± 9.98 b	22.10 ± 1.03 a	3.86 ± 0.15 c
ANOVA	*	*	*	*	*

Different lowercase letters in a column indicate significant differences between means for P ≤ 0.05 by LSD test

The ‘Kožara’ and ‘Kiseljaja’ cultivars had the longest fruit. Fruit width ranged from 79.57 ± 2.90 mm in the ‘Kiseljaja’ cultivar to 95.05 ± 3.71 mm in the Kožare cultivar, but the differences were not significant. Our results are consistent with the results found by Radunić et al. (2011) and Đorđević et al., (2013) in ‘Idared’ and Tomić et al. (2011) in ‘Kolačara’.

Fruit weight is a very important quality trait that influences both yield and consumer acceptance (Durmaz et al., 2010). In terms of fruit weight, the ‘Kožara’ cultivar had the highest fruit weight and the ‘Kiseljaja’ cultivar the lowest.

Several authors dealt with testing the fruit weight of apples. Vujević (2016) cites the fruit weight values of the ‘Kolačara’ cultivar, which were lower than in our study. This deviation can be attributed to the age of the orchard, as in the research conducted by Vujević (2016), the orchard is over 50 years old. However, the studies of other authors such as Gvozdenović et al. (1991), Đorđević et al. (2013) and Milatović et al. (2012) are similar to our results.

The highest flesh firmness was found in the ‘Kožara’ cultivar, the lowest in the ‘Kiseljaja’ and ‘Idared’ cultivars. These results do not agree with the results of Radunić et al. (2011), who measured higher penetrometer values for ‘Idared’, which could be due to the different harvest times.

Regarding the results of fruit petiole length, which can be very short, short, medium long, long and very long (Adamič, 1963), the 'Idared' and 'Kožara' cultivars had the highest values and the 'Kolačara' cultivar the lowest. These values are similar to the results found by Čmelik et al. (1999).

Table 4. Fruit phisical characteristics of defferent apple cultivars

Cultivar	Arithmetic mean diameter (mm)	Geometric mean diameter (mm)	Sphericity	Length/Diameter ratio	Surface area (cm^2)
'Kožara'	88.5 ± 0.01 a	90.46 ± 0.05 a	1.10 ± 0.00 c	1.15 ± 0.01 b	256.97 ± 3.20 a
'Kolačara'	77.11 ± 0.02 b	80.81 ± 0.02 b	1.30 ± 0.01 a	1.49 ± 0.02 a	205.07 ± 2.97 b
'Budimka'	75.58 ± 0.01 b	78.69 ± 0.05 b	1.23 ± 0.01 b	1.37 ± 0.02 a	194.46 ± 1.85 b
'Kiseljaja'	76.52 ± 0.02 b	77.48 ± 0.02 b	1.05 ± 0.00 d	1.08 ± 0.00 c	188.51 ± 1.56 b
'Idared'	75.26 ± 0.01 b	77.35 ± 0.01 b	1.13 ± 0.02 c	1.21 ± 0.07 b	187.91 ± 1.40 b
ANOVA	*	*	*	*	*

Different lowercase letters in a column indicate significant differences between means for $P \leq 0.05$ by LSD test

The 'Kožara' cultivar achieved significantly higher values for arithmetic and geometric fruit diameter as well as higher sphericity than the other cultivars. The best sphericity was found in 'Kolačara'. 'Kolačara' and 'Budimka' cultivars had the longest fruits, while the fruits of 'Kiseljaja' were the least elongated (Photos 1-4).

Photo 1: 'Kožara'


Photo 2: 'Kolačara'

Photo 3: 'Kiseljaja'


Photo 4: 'Budimka'

Significant and consistent differences were found between the cultivars in terms of SSC and TA (Table 5).

The cultivar 'Kožara' had the highest SSC value. The other cultivars had significantly lower SSC values.

Table 5. Fruit chemical characteristics of different apple cultivars

Cultivar	SSC (°Brix)	TA (%)	RI
'Kožara'	17.70±0.90 a	0.17±0.01 a	104.12±5.01 b
'Kolačara'	12.62±0.55 b	0.07±0.01 c	180.28±9.22 a
'Budimka'	12.65±0.51 b	0.06±0.01 c	210.83±7.33 a
'Kiseljaja'	14.01±0.59 b	0.14±0.01 ab	100.07±5.65 b
'Idared'	12.25±0.50 b	0.11±0.01 b	111.36±9.32 b
ANOVA	*	*	*

Different lowercase letters in a column indicate significant differences between means for $P \leq 0.05$ by LSD test

Our results for SSC are similar to those of Radunić et al. (2011) and Bostan (2009). On the other hand, Božović et al. (2015) found higher SSC content in 'Kolačara' than in our orchard. Large differences in SSC are not only the result of genotypic characteristics, and maturity stage but also depend on environmental factors and geographical region (Asif et al. 2004).

It is known that acidity plays an important role in the perception of fruit quality, as acidity balances fruit flavor (Schmitzer et al., 2011) and high acidity often reduces fruit quality.

The highest total acid content was found in the 'Kožara' and 'Kiseljaja' cultivar (Table 5). The lowest contents were found in 'Budimka' and 'Kolačara' cultivars.

Similar studies were carried out by Kołodziejczyk et al. (2009) and Radunić et al. (2011). In their studies, the 'Idared' cultivar had a slightly higher total acid content (0.14%) than in our study. In their research, Hecke et al. (2006) found that old apple cultivars in organic production in Austria have a higher content of total acids (from 8.3 g kg^{-1} to 17.85 g kg^{-1}) than commercial cultivars. It seems that the geographical region with its specific weather conditions also plays an important role in the biosynthesis of compounds that determine TA.

The ratio between SSC and TA (SSC/TA ratio or RI) plays an important role in consumer acceptance of fruit (Crisosto et al., 2005). In the current study, the highest RI value was observed in 'Budimka' and 'Kolačara' (Table 5).

Conclusion

The autochthonous cultivars generally showed good results and some of them even showed better characteristics than the control cultivar 'Idared'. Particularly noteworthy is the 'Kožara' cultivar, which showed the most positive characteristics. However, all cultivars showed relatively good characteristics, so they can be recommended for cultivation, especially for organic production, but also for the preservation of genetic material to obtain new cultivars for breeding.

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PRODUCTION AND TECHNOLOGICAL CHARACTERISTICS OF THE PROKUPAC WINE VARIETY IN THE NIŠ WINE-GROWING REGION

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Abstract: The paper presents the results of the study of some production and technological characteristics of the Prokupac wine variety in the Niš win-growing region. The research was conducted in the collective vineyard of the "Center for Viticulture and Oenology" in Niš. During the study period, the conditions were favorable for the expression of the production and technological characteristics of the Prokupac wine variety. The level of yield and its fluctuations from year to year indicate a high and stable fertility of the studied variety. The sugar content in the grape must was 21.58% and the total acidity 7.61 g/L. The chemical analysis of the wine showed that the wine obtained was of good quality. The results of examination confirm that the cultivation of the Prokupac wine variety in the Niš wine-growing region is justified.

Key words: phenological observations, bearing capacity, grape yield, grape and wine quality.

Introduction

The Prokupac wine variety is the most important autochthonous Serbian grapevine variety. In addition to Serbia, it is also cultivated in the Republic of North Macedonia and in Bulgaria.

It can be found under synonyms: Kameničarka, Prokupka, Crnina, Crnka, Rskavac, Niševka, Zarčin, Skopsko Crno, etc.

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Prokupac is an old autochthonous grapevine variety that was much more important in the past than it is today. In recent years, however, this variety has taken up significant areas in the assortment of the Republic of Serbia.

The aim of our paper was to verify the most important production and technological characteristics of the Prokupac wine variety in the conditions of the Niš wine-growing region.

Materials and methods

The research was carried out in the collection vineyard of the "Center for Viticulture and Oenology" in Niš. The vineyard belongs to the Niš wine-growing region. The altitude of the vineyard is 210 meters, the slope of the terrain is gentle and the exposure is north. The vineyard was planted in 1995 on an area of 2 hectares with over 200 grapevine genotypes.

The subject of this paper is the Prokupac wine variety, which was planted at a distance of 3.0 x 1.2 metres. The rootstock on which it was grafted is *Berlandieri* x *Riparia* Kober 5 BB. The training system was "Karlovački" with a tree height of 80 cm, using mixed pruning. The trellis support consists of a wooden stakes, concrete pillars and wire, which ensure the correct arrangement of shoots, leaves and bunches in space. During pruning on vine, one spur with 2 buds and two shoots with 10 buds each were left on the vine. In this way, all vines were loaded with the same number of buds, which eliminated the influence of the different loads on the vines on the yield and quality of the grapes.

The study was conducted in the period 2011-2013 and were divided into the field part of the trial and the laboratory chemical analysis of grapes and wine. The field part of the experiment included 10 vines per each trial treatment. The trial was designed using the completely randomised design method with ten replicates (10 vines) within a variant, with each vine served as a separate experimental unit. The laboratory analyses were conducted in the laboratories of the "Center for Viticulture and Oenology" in Niš, the Agricultural Extension Service in Niš and the Faculty of Agriculture in Belgrade.

The study included the following indicators: phenological observations, bearing capacity, yield and mechanical composition of the bunch, grape quality, chemical composition of the wine and sensory evaluation of the wine.

During the study period, the climatic conditions were favorable, as can be seen from the data in Table 1.

Table 1. Values of the basic climate indicators

Serial num.	Indicator	Year			Average
		2011	2012	2013	
1	Mean annual air temperature in °C	12.20	13.10	13.30	12.80
2	Mean vegetation temperature in °C	18.60	20.10	18.90	19.20
3	Annual amount of precipitation in mm	411.10	631.20	581.80	541.30
4	Precipitation in vegetation period in mm	273.00	370.20	297.30	313.15

The thermal conditions in this wine-growing area are favorable for the cultivation of vines and allow the normal ripening of grapes at all ripening epochs. In 2011 and 2013, the annual and vegetation temperature values were slightly higher than in 2012. In 2011, there was a drought during the vegetation period. In contrast, in 2012, precipitation was frequent and plentiful.

Results and discussion

Phenological observations

The timing of the individual phenophases in the annual development cycle of the Prokupac wine variety can be seen from the data listed in Table 2. The budburst occurred slightly earlier in 2011 and 2013 (April 12) and slightly later in 2012 (April 17). Flowering began on average 48 days after budburst (June 1). An average of 172 days passed between budburst and the first harvest. The first grape harvest was on October 1 at the earliest and October 5 at the latest. The veraison appeared on average (August 4) and the full ripeness of the grapes (October 3). Similar values are given by Zirojević (1974) for the conditions of the Niš wine-growing region and Garić et al. (2019) for the conditions of the Toplica wine-growing region.

Table 2. Phenological stages of the Prokupac wine variety

Year	Phenological phase						
	Budburst	Flowering		Berry develo pment	Verai son	Ripenig time	Numb er of days
		Beginning	Ending				
2011	12.04.	30.05.	14.06.	20.06.	03.08.	01.10.	173
2012	17.04.	03.06.	17.06.	23.06.	05.08.	05.10.	171
2013	12.04.	02.06.	15.06.	20.06.	03.08.	02.10.	173
Average	14.04.	01.06.	15.06.	21.06.	04.08.	03.10.	172

Bearing capacity

Based on the data shown in Table 3, it can be concluded that the highest values of all bearing capacity potential coefficients were recorded in 2013. The lowest values of the coefficient of potential and relative bearing capacity were in 2011. The highest absolute bearing capacity rate was in 2013 and amounted to 1.5, the relative bearing capacity rate was slightly lower (1.2) and the potential bearing capacity rate was the lowest (1.1).

Table 3. Coefficients of bearing capacity of the Prokupac wine variety

	Year	Coefficient of the potentional bearing capacity	Coefficient of the relative bearing capacity	Coefficient of the absolute bearing capacity
Prokupac wine variety	2011	0.8	0.9	1.4
	2012	0.9	1.0	1.2
	2013	1.1	1.2	1.5
	Average	0.93	1.03	1.36

Yield and quality of grapes

Based on the analysis of the data shown in Table 4, the following can be concluded. The yield of grapes per vine depended on the number of bunches per developed and fertile shoot, as well as on the size and weight of the bunches. The highest yield of grapes per vine was achieved in 2013 (4.50 kg), and slightly lower in 2011 (4.35 kg). The yield of grapes per hectare was calculated and amounted to 12,246.57 kg/ha. The largest bunch mass was achieved in 2013 (283.30 g), and the lowest in 2011 (205.68 g). The average length of the bunch was 15.87 cm and the width of the bunch was 9.10 cm. Similar values for the Prokupac wine variety are given by Zirojević (1974), Marković et al. (2013), Garić et al. (2019) and others.

Table. 4. Mean values and indicators of yield variability, grape quality and grape characteristics of the Prokupac wine variety

Characteristic	Year				Indicator of variability	
	2011	2012	2013	$\bar{X}$	S	Cv (%)
Grape yield per vine (kg)	4.35	4.49	4.50	4.41	0.95	21.49
Number of bunches per vine	18.1	21.4	18.2	19.23	2.80	15.08
Bunch mass (g)	205.68	240.90	283.30	243.29	66.42	27.30
Bunch length (cm)	15.7	16.2	15.7	15.87	1.83	11.55
Bunch width (cm)	9.2	8.9	9.2	9.10	1.75	19.22
The number of berries in a bunch	106.4	84.8	103.7	98.30	21.4	21.92
Mass of grape stalk (g)	7.00	6.22	4.80	6.01	1.98	33.01
The sugar content of grape must (%)	21.70	20.80	22.21	21.58	1.21	5.63
Content of total acids (g/L)	8.1	7.8	6.8	7.56	1.24	16.25
Alcohol content in wine (% Vol.)	12.96	12.71	13.10	12.92	/	/
Sensory evaluation of wine	60	63	61	61.33	/	/

The number of grape berries in a bunch varied from 84.8 to 106.4 g. The mass of the grape stalks ranged from 4.80 to 7.0 g. Also, it was determined that the yield of grapes per vine, the number of bunches per vine and the weight of the bunches varied within the studied variety (Cv=21.49, Cv=15.08, Cv=27.30).

Table 5. Mean values and indicators of variability of sugar content and total acids in the grape must of Prokupac wine variety

Characteristic	$\bar{X}$	S	C_v (%)
Sugar content in grape must (%)	21,58	1,21	5,63
Total acid content in grape must (g/L)	7,61	1,24	16,25

The data in Table 5 show that the sugar content of Prokupac grape must was 21.58% and the total acid content of grape must was 7.61 g/L. The variability, expressed by the coefficient of variation, was low for both characteristics.

Table 6. Statistical significance of the influence of genotype and year on the sugar and total acid content of grape must of the Prokupac wine variety

Sources of variation	The sugar content in grape must		The content of total acids in grape must	
	ANOVA			
	F-value	*p-value*	*F-value*	*p-value*
Genotype	34.90^{**}	0.00	3.03^{nz}	0.05
Year	3.53^{*}	0.03	0.21^{nz}	0.81
Genotype x Year	4.00^{**}	0.005	3.60^{*}	0.01
DUNNETT-*test*				
Prokupac	21.58^{**}		7.61^{nz}	

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

The results of the analysis of variance in Table 6 show that the influence of the year was not significant for the sugar content in the grape must and also not for the content of total acids in the grape must (p> 0.05). The joint influence of genotype and year was very significant for the sugar content in the grape must and significant for the content of total acids in the grape must.

Conclusion

Based on the results of the study on the production and technological characteristics of the Prokupac wine variety in the Niš wine-growing region, the following conclusions can be drawn:

In the agro-ecological conditions of the Niš wine-growing region, there are favorable conditions for the normal development of the Prokupac wine variety and the achievement of high-quality grapes and wine.

The Prokupac wine variety had the earliest budbust on April 12, the beginning of flowering on May 30 and grape ripening on October 1.

The coefficients of potential, relative and absolute bearing capacity as indicators of cropping potential showed the highest values for the studied variety.

The highest yield of grapes per vine (4.50 kg) was achieved in 2013, while the highest number of bunches per vine was recorded in 2012 (21.4).

The quality of the grapes, assessed on the basis of the sugar and total acid content in general and the health of the grapes, can be classified as very good.

The sugar content of the grape must was between 20.80 and 22.10 % and the total acidity between 6.8 and 8.10 g/L.

The sensory evaluation of wines in the Prokupac wine variety was from 60 to 63 points.

From the results of the study of the production and technological characteristics of the Prokupac wine variety under the conditions of the Niš wine-growing region, it can be concluded that the Prokupac wine variety can be successfully cultivated in this region.

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THE INFLUENCE OF SANITARY STATUS AND THE POSITION OF EXPLANTS ON THE GROWTH AND DEVELOPMENT OF IN VITRO OF 'PLAVAC MALI' AND 'POŠIP'

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Abstract: This study determines the *in vitro* influence of viruses (GLRaV-1, GLRaV-3, GFLV, GVA) and the position of meristems on initial shoots on the growth and development of 'Plavac Mali' and 'Pošip' grapevine cultivars. Infected clones were inoculated on MS medium with 0.5 mg l^{-1} benzylaminopurine (BAP) and 0.05 mg l^{-1} indole-3-acetic-acid (IAA). The positions of meristems were: a – 1st; b – 2nd and 3rd; and c – 4th and 5th. The height and number of nodes, as well as the size of callus, were measured four and eight weeks after inoculation. Clone, position of explants, and sanitary status show no significant influence on tested parameters, except in callus formation, where shoot apex formed the lowest callus. Results revealed that axillary buds on the shoot of grapevine, infected with economically important viruses, can be used for biotechnology purposes.

Keywords: grapevine, multiplication of clones, virus infection, position of buds

Introduction

Grapevine represents a significant crop in agricultural production in Croatia. In a population of Croatian autochthonous cultivars, a high infection of economically harmfull viruses has been determined (Čarija et al., 2022). In the production of a healthy plant material, methods of rapid multiplication and virus eradication *in vitro* can be successfully applied. Cultivars 'Plavac Mali' and 'Pošip' represent important cultivars for the Dalmatia wine region in Croatia, as well as for Croatian viticulture in general (Figure 1). Individual clonal selection was performed on both cultivars, and the registered clones are

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cultivated in the national pre-base genebank. Both cultivars ‘Plavac Mali’ and ‘Pošip' were previously included in tissue culture research activities, and their *in vitro* adaptibility was the main reason they were selected for this research (Marković et al., 2014).

The aim of this study is to determine the influence of infection with economically important viruses (Grapevine leafroll associated virus-1, Grapevine leafroll associated virus-3, Grapevine Fanleaf virus, Grapevine virus A) and the position of buds on shoots used for establishment of aseptic culture on the growth and development of ‘Plavac Mali’ and ‘Pošip’ varieties introduced in *in vitro* meristem culture.

Figure 1. ‘Plavac Mali’ and ‘Pošip' cultivars

Materials and methods

Buds used for the establishment of an aseptic culture *in vitro* were taken from initial shoots collected from infected clones of 'Pošip' and 'Plavac Mali' cultivars that have been selected from the National Collection of Autochthonous Cultivars. The wooden cuttings were placed in a water in a room conditions. Buds/meristems were categorized based on their position on the initial shoot: a - apical meristem; b – 2^{nd} and 3^{rd} meristem; and c – 4^{th} and 5^{th} meristem. The inoculation was made with meristems 1-2 mm in size, each containing two primordial leaves. Murashige and Skoog (1962) medium (MS) supplemented with 1mg l^{-1} benzylaminopurine (BAP) and 0.05 mg l^{-1} indole-3-acetic-acid (IAA) was used for initiation of aseptic cultures.

In a period of four weeks, the growth and development of the explants was monitored, followed by measurement of the height and the number of explant nodes (Figure 2). During the monitoring of the development, uneven growth and development, as well as the formation of callus, were observed on the majority of explants, so the explants were subcultured on standard MS medium after measurements.

During subcultivation, leaves and calli were removed from the explants to promote growth for the next four weeks until the next scheduled measurement. After eight weeks, the growth and development of the explants was monitored, and a second measurement of shoot height and the number of nodes, as well as the presence of calli was performed to assess the effect of both variety and sanitary status on growth and development *in vitro*.

All parameters (shoot height, number of nodes, and the size of callus) were measured after four and eight weeks of inoculation. Data processing by one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test were performed by XLSTAT program (2007).

Figure 2. Measurement of height, number of nodes and callus 4 weeks after inoculation

Results and discussion

There are significant differences in growth *in vitro* between cultivars, revealing that all clones of 'Pošip' differ from all clones of 'Plavac Mali' (Table 1). The basis of this is the genetic background of cultivars. Within cultivars there are no significant differences between clones. Contrary to our expetations, slighty lower results were obtained for virus-free clones in all investigated parameters. This differs from previous findings on the cultivar 'Plavac Mali', where the healty clones outperformed the infected ones (Marković et al., 2014). However, selecting the right phenological stage for introducing explants *in vitro* can imrove the growth of infected clones/cultivars (Marković et al., 2021).

Table 1. The influence of the clone on the growth and development of the explants of 'Plavac Mali' and 'Pošip' cultivars *in vitro*

Clone	Height	No.of nodes	Callus
POŠ 68-4 (GVA)	2.967 a	3.333 a	1.273 a
POŠ (virus-free)	2.932 a	3.421 a	1.268 a
POŠ 89-11 (LR1 +LR3)	2.411 a	3.053 a	1.084 ab
PMC19-15 (GFLV)	1.390 b	1.300 b	0.365 c
PMC 19-2 (LR1)	1.300 b	1.200 b	0.680 c
PMC (virus-free)	1.220 b	0.600 b	0.700 bc
Pr > F(Clone)	<0,0001	<0,0001	<0,0001

Mean values in each column followed by different letters are significantly different according to Duncan's Multiple Range Test, $P \leq 0.05$

The position of buds on the initial shoot was not significantly related to plant height or number of nodes, however it was significant for callus occurence (Table 2). The upper positions of buds on the shoot are more sensitive to callus formation. Inoculation medium should also be revised for callus sensitive cultivars. Using a hormon-free media is risky for initial *in vitro* cultivation since the buds require some stimulation to begin growing. However, this study demonstrated that for assessing tissue culture growth, more than just the shoot apex can be used. That makes the procedure for introducing in tissue culture conditions more flexible.

Table 2. The influence of the category of meristem on the initial shoot on *in vitro* growth of cultivars 'Plavac Mali' and 'Pošip'

Category of meristems	Height	No.of nodes	Callus
c	2.185	2.341	1.102 a
b	2.143	2.429	0.893 a
a	1.620	1.550	0.450 b
Pr > F (category of meristems)	0.180	0.136	0.001

Mean values in each column followed by different letters are significantly different according to Duncan's Multiple Range Test, $P \leq 0.05$. If the values are not followed with small letters at all, they were not significantly different. a - apical meristem; b – 2nd and 3rd meristem; and c – 4th and 5th meristem

There was no significant difference in growth *in vitro* of the studied cultivars between healthy and infected shoots (Table 3), as well as between those with individual infection (GVA, GFLV, GLRaV-1) and mixed infection with viruses (GLRaV-1+GLRaV-3) (data not shown). This implies that all infected genotypes can be grown in tissue culture and then subjected to biotechnological metods for virus eradication.

Table 3. The influence of the sanitary status on the growth *in vitro* of cultivars 'Plavac Mali' and 'Pošip'

Category of infection	Height	Nbr.of nodes	Callus
VF (virus-free) clones	2.341	2.448	1.072
Infected clones	1.947	2.135	0.819
Pr > F (Sanitary status)	0.129	0.401	0.074

There were no statistically significant differences between means as determined by one-way ANOVA

Conclusion

Tissue culture is effient tool for establishing healthy clones of Croatian autochthonous cultivars. However, since some cultivars do not respond well to tissue culture, it is adviseable to test traditional propagation methods. In contrast to common practice, the highest growth *in vitro* was observed in buds on the second and third positions from the apical meristem, as well as the highest number of nodes with buds on the first position. No specific sanitary virus status is deleterious in tissue culture conditions. Thus, *in vitro* population establishment for sanitation programmes could be designed with an axillary bud and all types of virus infections from vineyards, as well as decreased concentrations of BAP in the inoculation medium due to calli occurence.

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INFLUENCE OF DIFFERENT VINEYARD WEED CONTROL METHODS ON AGROBIOLOGICAL, ECONOMICAL AND TECHNOLOGICAL CHARACTERISTICS OF VRANAC VARIETY

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Abstract: The study of the impact of different methods of weed control on the agrobiological, economical and agrotechnological characteristics of the Vranac variety was carried out in the Podgorica vineyard in 2015. Six different variants of weed control were applied: control, mechanical weed control, glyphosate (one treatment), glyphosate (two treatments), flazasulfuron and flazasulfuron + glyphosate.

The yield of grapes during the research period ranged from 2.9 kg/vine in control to 3.9 kg/vine in the variant with combined use of flazasulfuron and glyphosate. The lowest average cluster weight was measured in the control - 169 g, while the highest was measured in the variant with the application of flazasulfuron + glyphosate - 206 g. The lowest sugar content was found in the control variant - 23.8%, and the highest in the variant with mechanical weed control - 25.8%. Varieties with one and two treatments with glyphosate had the most acids in the must - 6.43 gl^{-1}, and the variant with mechanical weed control had the lowest acid content in must - 5.57 gl^{-1}.

Keywords: Vranac, weed control, cluster weight, grape yield and quality

Introduction

The red wine variety Vranac has great economic importance for Montenegro and dominates the grape assortment. This variety is characterized by low ecological plasticity, and for these reasons it produces grapes of lower quality outside Montenegro (Savić, 2016).

Weed control in the vineyard is a permanent agrotechnical measure based on regular autumn, spring and summer tillage, weed control in surrounding

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areas, prevention of flowering and seed production, use of natural or synthetic mulches and application of herbicides (Mirošević, 2008). Due to the pronounced lack of manpower and the high costs of mechanisation usage, the application of herbicides in conventional viticulture is of great importance, due to its effectiveness, timely and long – lasting protection against weeds (Kovačević, 2008). However, unprofessional application of herbicides often leads to a phytotoxic and depressing effect on the vine, which results in yield and quality decrease of produced grapes, as well as resistance to the most dangerous diseases (Marković, 2012). In addition, unprofessional application of herbicides can have a negative effect on the soil and the surrounding environment, endangering beneficial flora and fauna (Jovović et al., 2013).

Hence, this study was designed with the aim to discover the most effective weed control method in the conditions of the Podgorica vineyard that will enable achievement of high quality and satisfactory yields.

Materials and methods

The study of the effectiveness of different methods of weed control on the biological, economical and technological characteristics of the Vranac variety was carried out in the experimental vineyard of the Biotechnical Faculty in 2015. The Vranac variety was grafted on Berlandieri x Riparia Kober 5BB rootstock, and the vineyard was planted with a planting distance of 2.4 x 1 m in 2005. The cultivation form is a two rods horizontal cordon with a stem height of about 80 cm. Tests were carried out on 180 vines, in three repetitions with 10 vines in each variant. The selected vines were pruned according to the same criteria, using mixed pruning, with eight buds on each of two canes and two buds on each of two spurs, resulting in load of twenty buds per vine plant.

The following weed control variants were studied: control, no weed control (K), mechanical weed control (tillage between and within the vine rows, MO), Glyphosate 480 SL, one treatment at the rate of 4 lha^{-1} (H1), Glyphosate 480 SL, two treatments with the rate of 2 lha^{-1} each (H2), Chikara 25 VG, one treatment before weed emergence in the spring, in the amount of 200 gha^{-1} (H3) and Chikara 25 VG (200 gha^{-1}) + Glyphosate 480 SL (4 lha^{-1}) (H4).

The following parameters were studied: grape yield (weighing the harvested grapes from each vine plant), cluster weight (the ratio between the yield and the number of clusters), the sugar content in the grape juice (using Oechsle's refractometer) and the proportion of total acids in the grape juice (neutralization of all acids and their salts with n/10 NaOH solution with bromthymol blue as indicator).

The average annual air temperature was 17.2 ^{0}C and the average vegetation temperature was 23.2 ^{0}C n the Podgorica subregion in 2015. The sum of the effective temperatures was very high, and amounted to >2300 ^{0}C. Total of 1176.0 l/m^2 of precipitation was measured in the studied year, whereas only 438 l/m^2 of rain fell during the vegetation season.

Statistical data analysis was performed by analysis of variance for a completely random block design system. Significance of differences was determined using the LSD test for pairwise comparisons at significance levels of 0.05 and 0.01, respectively.

Results and discussion

The results presented in Tab. 1 show that the control variant had the lowest cluster weight (169 g) in this study. The highest cluster weight was measured in the variants H4 - 206 g and H3 - 200 g. All variants with the application of herbicides had a statistically higher average cluster weight compared to the control. The results of this research showed that the cluster weight was at the level of the values reported for the Vranac variety by Pejović (1982), Matijašević (2021), and Popović (2021).

Table 1. Average cluster weight and yield of Vranac variety

Variant	Cluster weight (g)	Grape yield (kg/vine)
K	169	2.9
MO	184	3.0
H1	191	3.1
H2	193	3.5
H3	200	3.6
H4	206	3.9

	LSD 0.05	LSD 0.01
Cluster weight	19.238	26.971
Grape yield	0.3880	0.5440

The obtained results show that the yield of grapes per vine varied significantly between the studied treatments. The highest yield of grapes (3.9 kg/vine) was measured in the variant H4, followed by treatments H3 and H2

(3.6 and 3.5 kg/vine, respectively). The lowest yields were measured in vine plots without weed control (2.9 kg/vine), as well as on those where mechanical weed control measures applied - 3.0 kg/vine. Statistical data analysis showed a very significant increase in yield in the H4 variant compared to the control and herbicide treatments H2 and H3, respectively and only significantly higher compared to the H1 variant. Grape yields obtained in this research are in agreement with the results reported by Pejović (1982) and Popović et al. (2012) for Lješkopolje conditions.

The content of sugar and acids in the grapes is one of the basic indicators of grape quality. Their content in grape juice is influenced by numerous factors, first of all the variety, then the ecological conditions during the year, the degree of ripeness and the health condition of the grapes (Cindrić, 2000). From the data presented in Table 2, it can be concluded that the sugar content in the must varied significantly depending on the method of weed control.

Tab. 2. Content of sugar and total acids in grape juice

Variant	Sugar content (%)	Acid content (g/l)
K	23.8	5.8
MO	25.8	5.6
H1	25.4	6.4
H2	24.4	6.4
H3	25.0	6.3
H4	25.5	6.3

	LSD 0.05	LSD 0.01
Sugar content	1.1157	1.5642
Acid content	0.4612	0.6466

The highest must sugar content was found in the variant with mechanical weed control - 25.8%. The variant with the combined application of the Chikara + Glyphosate herbicide had a slightly lower sugar content - 25.5%. A high sugar content was also recorded in the variants H1 - 25.4% and H3 - 25.0%. The lowest amount of sugar was measured in the control - 23.82% and variant H2 - 24.4%. Statistical data analysis showed a statistically very significant difference in the content of sugar in must between the variants MO, H4 and H1 and the control. The difference between the variants MO and H4 compared to the variant H2 was considered significant. The content of sugar in the must in the experiments was within the limits stated for the Vranac variety by other authors: Božinović (2010), Pajović (2014), Maraš (2018) and Popović (2021).

The content of total acids in the grape juice as a whole was satisfactory and characteristic for the Vranac variety grown in the agro-ecological conditions of the Podgorica vineyard. The variants H1 and H2 had the highest acid content - 6.4 gl^{-1}, while the lowest content was in the variant in which weeds were controlled mechanically - 5.6 gl^{-1}. Statistical data analysis revealed a very significant difference in the content of acids in the must between all studied treatments and the MO variant and significant in comparison with the control. Similar data on the content of total acids in Vranac must in the Podgorica subregion were also reported by Raičević et al. (2015).

Conclusion

Based on the results of the study of the impact of different methods of weed control on the agrobiological, economical and technological characteristics of the Vranac variety in the Podgorica vineyard, the following is concluded:

- The highest grape yield in the research was in the variant with the combined application of Chikara + Glyphosate herbicides (3.9 kg/vine), while the lowest yield was recorded in the control variant (2.9 kg/vine).
- In all applied methods of weed control, the average cluster weight was higher compared to the control. The largest cluster weight was measured in the variant with the combined application of Chikara + Glyphosate (206 g).
- The highest sugar content in must was measured in the variant with mechanical weed control (25.8%), but the H4 (25.5%) and H1 (25.4%) variants also had a very high sugar content. The lowest sugar content was found in the control (23.8%).
- Variants H1 and H2 had the highest acid content in grape juice (6.4 gl^{-1}), while the lowest content was measured in the variant with mechanical weed control (5.6 gl^{-1}).

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CALCULATIONS IN RASPBERRY PRODUCTION ON FAMILY FARMS IN WESTERN SERBIA

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Abstract: The area of Western Serbia has the highest average production of raspberries and a long tradition and is also recognizable for the quality and export of raspberries. On the family farms of this hilly and mountainous area, raspberry production takes place in small areas with a large labour participation. The research aims to show the production on the average family farm through economic analysis and solve the problem of labour shortage by producing raspberries in an area of 0.2 ha. Based on real data from several such farms, the most important economic parameters were calculated and shown in the calculation of raspberry production. The gross margin with the average raspberry purchase price of €1.8 was €1,300. By calculating the critical values of prices, yields and variable costs, as well as based on a sensitive analysis of the gross margin, the risks and profitability of investing in raspberry plantations were indicated.

Keywords: raspberries, costs, gross margin, purchase prices

Introduction

For years, raspberry production in Serbia has been geared towards exports to EU countries, where there is a high demand for this fruit, which increases the inflow of foreign currency and at the same time contributes to the income of farmers and the local economy. Favorable climate and soil conditions have contributed to the fact that high-quality raspberries have been produced in the raspberry-growing region of western Serbia for many years (Petrović i Leposavić, 2011).

The economic importance of raspberry production is determined by the following factors: high production value, income, and profit per unit of invested capital, production is labor-intensive and affects the additional employment of labor, there is a high return on investment under favorable agroecological and economic conditions, which is confirmed by the research of

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Sredojević et al. (2013), Kljajić et al. (2017). Family farms in these areas are often in cooperative relationships with cold stores that buy their raspberries and accordingly apply adequate production technology to achieve the required quality of raspberry fruits (Veljković et al. 2006).

The economic importance of raspberry production in the hilly and mountainous areas of Serbia is that it contributes to rural development, and has a positive effect on the diversification of agricultural production and employment of the population. It also contributes to the development of entrepreneurship on family farms and the connection with the food industry and tourism.

Materials and methods

The research provided a concise overview of raspberry production in Serbia, encompassing key parameters such as production volume, yield, and purchase prices. It also highlighted the volatility of these parameters throughout the period from 2013 to 2023. Official databases from the Statistical Office of the Republic of Serbia were utilized for statistical analysis.

In the economic analysis, calculations grounded in variable costs were employed, and the gross margin was determined as the difference between the production value and the variable costs of raspberry production (Jeločnik et al., 2021). The production value hinges on both achieved yields and the purchase price of raspberries. Variable costs encompassed material expenses, labor expenditures, and mechanization costs within the raspberry orchard. Variable costs included material costs, labor costs, and mechanization costs in the raspberry orchard. The analysis encompassed the calculation of the gross margin and sensitivity analysis, as well as an evaluation of the return on investment. The calculation used data from family farms that have many years of experience in raspberry production and are located in the area of Western Serbia (Arilje, Ivanjica, Dragačevo, Kosjerić). By evaluating parameters like the gross margin, the analysis aimed to ascertain the efficacy and success of raspberry production.

Results and discussion

Based on the analysis of raspberry production in Serbia in the period 2013-2023. year, it can be concluded that the year of highest production was 2018 with 127010 t, in the same year the lowest purchase price for raspberries was 0.9

€. The highest purchase price for raspberries of € 4.2 was achieved in 2022, and the yields at that time were 5.5 tha^{-1}. The highest raspberry yields of 6 tha^{-1} were achieved in 2015, and the lowest of 4.9 tha^{-1} in 2020, at the same time the raspberry area was the largest in that year with 24028 ha. The lowest production and acreage of raspberries was recorded in 2013 (see Table 1). From the production parameters analyzed in Table 1 for the observed period, the coefficients of variation of the CV (CV) were calculated, from which it can be concluded that the lowest fluctuations in yields are 6.6% and the extremely high fluctuations in raspberry purchase prices are 54%. Instability in raspberry purchase prices increases risks and uncertainty in the final production outcomes. According to research by Milić et al. (2019) in the period 2008-2017 year, the coefficient of variation of raspberry purchase prices was 25.85%.

Table 1. Raspberry production parameters in Serbia in the period 2013-2023.

Parameters of production	Area in ha	Production in t	Yield tha^{-1}	Purchase prices €
The average	19603	106231	5.5	1.8
Max	24028	127010	6	4.2
Min	13118	74682	4.9	0.9
CV in %	18.1	15.3	6.6	54

Source: Calculated by authors, data of the Statistical Office of the Republic of Serbia

There are many farms growing raspberries in the raspberry regions of Western Serbia. The suitable agro-ecological conditions in this area have influenced that family farms in this production have many years of experience twenty or more years. Raspberry production is often an additional business that family farms pursue to use their resources more efficiently (Veljković et al. 2006). This production also requires greater use of human labour in certain phases of raspberry vegetation, especially during harvest. Since it is a labour-intensive production and the seasonal labour problem increases, the average family farms remain in smaller areas of up to 0.5 ha for raspberry production. Good results in terms of yield and quality of raspberries are achieved at an altitude of over 400 m, the most common variety being Willamette (Petrović i Leposavić, 2011). Raspberry production on 0.2 ha can be successfully realized by a four-person family farm with its labour with minimal participation in the cost of additional labour. In the economic analysis of raspberry production on the family farm, the costs of planting raspberries in an area of 0.2 ha are also taken into account. The calculation of the costs of planting and caring for raspberries was carried out at prices for the period 2021-2023. It should be emphasized that the years 2021 and 2022 were extremely favourable in terms of purchase prices for raspberries compared to the average purchase prices of the last 10 or more years. Producers who raised raspberry plantations in that period had a return on their investment already in the first year. To more realistically look at the economic results of this production in a longer period in the calculation of income, the average purchase price of raspberries of €1.8 was taken (Table 1). The increase in yield (income) and costs was monitored for three years, and according to the results, the investment in raising orchards was paid off in the third year of production (Table 2).

Table 2. Investment profitability costs and income in a raised raspberry orchard

	Cost €		**Income €**		**Balance €**
	Per years	**Cumulatively (A)**	**Per years**	**Cumulatively (B)**	**B-A**
Raising orchard	3544	3544	-	-	**-3544**
I year	2400	5944	3060	3060	**-2884**
II year	2660	8604	3960	7020	**-1584**
III year	2660	11264	4500	11520	**+256**

Table 3. Calculation of the gross margin of raspberry orchard on 0.2 ha

A. Production value (in the second year)				
Product	Unit	Yield	Price €	Amount €
Raspberry	kg	2200	1.8	**3960**
B. Variable costs				
Fertilizer				280
Pesticides				310
Machine work				390
Work of workers				330
Harvest				1200
Transport				60
Others costs				90
Total costs				**2660**
Gross margine = A-B				**1300**

The calculation of production during the growing season and the care of raspberries is shown in Table 3. In the calculation of the realized value of production during the year, on the family farms in this area average yields of raspberries achieved 11 t ha^{-1}. Considering that the cost values were collected from several farms in different periods, the average costs were calculated and their values were rounded.

In variable costs, the largest share is the work of workers and raspberry picking, 45%. Production technology and the calendar of works performed in the raspberry cultivation were monitored during the growing season. The farm members did all the work in the raspberry cultivation, from fertilizing and feeding about four times during the growing season, cultivating, tying shoots, pruning, and protecting against weeds, diseases and pests about five treatments. Raspberry harvesting, which is also the most sensitive part of this production, was also mostly done by farm members, which was feasible in this area. On average, a worker harvests 50-60 kg of raspberries per working day, and depending on weather conditions and altitude, the harvest lasts about 25-30 days. Based on the calculation in Table 3, a gross margin of €1,300 was achieved for raspberry production in an area of 0.2 ha at an average purchase price of €1.8. It should be emphasized that the members of the farm did all the work at cultivation and that the need for external services was minimal; the labour and harvesting costs remained largely with the farm. All this indicates that the family farms find an economic calculation and remain in raspberry production. In addition, the farms are very cautious when deciding to increase the area due to the shortage of labour.

Table 4. Sensitive analysis of gross margin in raspberry production

Expected values GM		€/kg	*-20%*	*-10%*	Price	*+10%*	*+20%*
			1.44	1.62	**1.8**	1.98	2.16
kg/ 0.2 ha	*-20%*	1760	-125.6	191.2	508	824.8	1141.6
	-10%	1980	191.2	547.6	904	1260.4	1616.8
	Yield	**2200**	508	904	**1300**	1696	2092
	+10%	2420	824.8	1260.4	1696	2131.6	2567.2
	+20%	2640	1141.6	1616.8	2092	2567.2	3042.4

Critical price = 2660 / 2200 = 1.2 €
Critical yield = 2660 / 1.8 = 1477 kg
Critical variable costs = 2200 x 1.8 = 3960 €

A sensitive analysis of raspberry production has shown how resistant the gross margin of realized production is to price and yield fluctuations that can occur in certain years, as previous experience has shown. The best-case scenario assumed in the sensitivity analysis is that the purchase price and yield increase by 20 %, which would have an impact on the increase in gross margin a 2.34-fold (Table 4). In the worst case, i.e. if the purchase price and yield were to fall by 20%, the gross margin would show negative values. To assess the stability of raspberry production, the critical price, critical yield, and critical variable costs were calculated. If the variable costs were to increase by 49% and the other parameters remained the same, the gross margin would be zero. The critical price is also the cost price of raspberries, i.e. the actual producer price, i.e. the price a producer has to pay for the production of 1 kg of raspberries.

The harvested raspberry fruits must be swiftly transported to refrigeration units for processing, necessitating meticulous organization and logistics. Simultaneously, there exists a significant reliance on purchase prices, with the gross margin in raspberry production exhibiting heightened sensitivity to fluctuations in raspberry purchase prices.

Conclusion

In previous analyses of raspberry production, a comprehensive review of the average economic parameters and outcomes associated with this production on family farms was conducted. Specifically, the stability and sensitivity of raspberry production on small estates were emphasized. Despite frozen raspberries being a significant export commodity from Serbia to EU countries, it is evident that there is still room for improvement in terms of organization and transparency within the production, processing, and export chain. Consequently, each segment of this chain is currently confronted with production and market risks individually. Raspberry producers are left with a dilemma and a guess as to what purchase price they can expect.

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COMPARATIVE ANALYSIS OF PRODUCTION AND SLAUGHTER PARAMETERS OF FAST-GROWING BROILER HYBRIDS

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Abstract: This study aimed to explore how hybrid varieties impact broiler chickens' production and slaughter performance. We applied standard rearing techniques over a 42-day period. The trial involved a total of 420 broiler chickens of the following hybrids: Cobb 500 and Ross 308. Weekly performance tests were conducted, assessing average daily gain, feed consumption, feed conversion rate, mortality, and European Poultry Efficiency Factor. After controlling for final body weights on the 42nd day, a random sample of 12 broilers per genotype, with an equal gender distribution, was selected for slaughter parameter analysis. Following slaughter traits were determined: pre-slaughter weight of chickens, slaughter yield, share of abdominal fat, chicken thigh circumference, and chest angle. The results revealed no significant difference in food consumption between the examined hybrids. Chickens of the Cobb 500 genotype exhibited significantly higher ($p<0.01$) gain and a more favorable feed conversion rate. Due to its better vitality, feed conversion rate, and gain, Cobb 500 chickens demonstrated significantly higher ($p<0.01$) European Poultry Efficiency Factor values. In contrast, Ross 308 chickens displayed statistically significantly lower ($p<0.05$) slaughter yield and chest angle values. At the same time, the proportion of abdominal fat and chicken thigh circumference showed no significant difference between the tested hybrids.

Keywords: broiler hybrids, production performance, slaughter parameters

Introduction

In the Serbian poultry industry, where domestic selection is absent, the evaluation of production and slaughter performances has been limited to foreign fast-growing hybrids designed for poultry meat production for the past thirty years or more. Comparisons of different hybrids, both domestically and

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globally, are standard practices, and the publication of the results from such comparisons typically attracts significant attention and sparks controversy. Various hybrids are used to produce poultry meat; among the most represented in our country are Cobb 500 and Ross 308. A comparative analysis of their production performance in our growing conditions is significant and interesting since it gives us the answer to the question of how much breeding centers in the world have progressed and improved certain production traits in relation to the previous period and other competing companies. Genetic advancements contribute to evident year-to-year improvements in production parameters (Marcos and Fausto, 2016). However, it's crucial to recognize that selection progress aimed at enhancing specific traits may inadvertently lead to the deterioration of other equally important characteristics. In the meat and slaughterhouse industry, there is a particular interest in optimizing the slaughterhouse performance of broilers. Consequently, as highlighted by Hristakiev et al. (2014), there is an ongoing focus on determining these crucial parameters. The quality of the chicken carcass can be evaluated from various aspects, including yield, meat composition, proportion, and content of abdominal fat in the carcass. Additionally, aspects related to the development of chicken breasts and thighs, as the most valuable carcass parts, are of significant importance.

Numerous factors can impact both the production performance and the mentioned quality parameters of chicken carcasses in broiler production. Several studies, including those by Pavlovski et al. (2009) and Lukić et al. (2020), have indicated that genotype can play a significant role. Petričević et al. (2011) further validate in their experiments that, among biological factors, genotype and gender exert the most substantial influence on carcass quality. The objective of our research was to investigate the impact of the more prevalent fast-growing hybrids in our market on production and slaughter performance.

Materials and methods

Sample material and diet

The experiment took place over a 42-day period at the Experimental Poultry Farm of the Institute of Animal Husbandry in Belgrade. Sample material consisted of 210 one-day-old broiler chickens each for the Cobb 500 and Ross 308 hybrids. The birds were housed in six boxes, with 70 chickens per box, and were distributed randomly using a block system to minimize potential environmental variations. Throughout the experiment, the chickens were provided with the same feed mixtures ad libitum.

A total of four pelleted mixes, purchased from the market, were utilized: Starter for the first two weeks, Grower for the third and fourth weeks, Finisher I for the fifth week, and Finisher II for the sixth week. The chemical composition and microbiological status of the mixtures were monitored in the accredited laboratories of the Institute. The analysis results validated the nutritional declarations by the manufacturers and affirmed the legally regulated quality and health status of the utilized mixtures. The crude protein content in the mixtures was 22.09%, 19.07%, 18.86%, and 17.24%, with calculated metabolic energy values of 12.9, 13.2, 13.5, and 13.8 MJ.

Determination of production and slaughter carcass traits

At the end of each week, the body weights of all broilers were measured, as well as the amount of food consumed. Food consumption, average daily gain, feed conversion, and mortality were monitored at the box level so that in the statistical processing of the data, the box represented the unit of observation. Based on data on body weight, feed conversion, and mortality, the value of the European Poultry Efficiency Factor (EPEF) was calculated according to the formula: EPEF = (Body weight (kg) x vitality (%) x 100)/(Duration of fattening (days) x feed conversion) (g/g)).

To assess carcass quality properties, following the final body weight check on the 42nd day, a random sampling method was employed to select 2 male and 2 female broilers from each box. This process formed a sample of 12 chickens per hybrid. After a 10-hour fasting period, a pre-slaughter body weight measurement was conducted just before slaughter to express relative values of slaughter traits. Post-slaughter and processing, carcasses were cooled and measured, with weights determined excluding poultry offal. The separation of abdominal fat occurred during carcass processing, representing fat tissue not linked to the carcass.

The obtained carcass and abdominal fat weights were then compared with the body weight of chickens before slaughter to determine the proportion of abdominal fat in the carcass. Additionally, within the scope of body conformation measures determined by the method outlined in Pavlovski et al.'s (2006) work, the circumference of chicken thighs at the widest part and the chest angle (expressed in degrees) were measured using a protractor vertically in relation to the back line.

Statistical data processing

Statistical analysis of the results was performed using the program package "STATISTICA" (Stat Soft Inc., 2012). A one-factor analysis of variance and an LSD test were used to determine the statistical significance of differences between mean values.

Results and discussion

At the outset of the test, the initial body weights of chickens were consistent and did not exhibit statistically significant differences between the Cobb 500 and Ross 308 hybrids (41.2g and 42.4g). Table 1 represents the production performance indicators of broiler chickens across the test periods, extending from the start of the experiment to the alteration of the feeding mixture.

Table 1. Production performance from the beginning of the test to the change in the feeding mixture

Period	Hybrid	Production parameters									
		Consumption (g/day)		Gain (g/day)		Conversion (g/g)		Mortality (%)		EPEF	
		x	Sd	x	Sd	x	Sd	x	Sd	x	Sd
1-14 days	Cobb 500	42.03	0.89	33.68^A	0.32	1.25^B	0.01	0.48	0.83	292.33^A	3.61
	Ross 308	40.65	0.90	30.69^B	0.54	1.32^A	0.02	0.95	1.65	254.19^B	4.84
	p-value	n.s.		<0.01		<0.01		n.s.		<0.01	
1-28 days	Cobb 500	85.02	0.78	59.64^A	0.11	1.42^B	0.01	0.95	0.83	425.34^A	5.52
	Ross 308	82.46	0.54	54.85^B	0.20	1.50^A	0.01	1.43	1.43	371.31^B	4.99
	p-value	n.s.		<0.01		<0.01		n.s.		<0.01	
1-35 days	Cobb 500	97.97	0.94	66.38^A	0.37	1.47^B	0.01	0.95	0.83	453.92^A	4.95
	Ross 308	99.24	0.44	63.28^B	0.12	1.56^A	0.01	1.91	0.83	405.65^B	4.29
	p-value	n.s.		<0.01		<0.01		n.s.		<0.01	
1-42 days	Cobb 500	112.76	2.00	70.56^A	0.15	1.59^B	0.02	1.43	1.43	443.67^a	13.44
	Ross 308	112.57	0.12	66.97^B	1.09	1.68^A	0.02	2.86	1.43	398.54^b	17.37
	p-value	n.s.		<0.01		<0.01		n.s.		<0.05	

x – average; Sd – standard deviation; n.s. - not significant

* a-b Mean values in each column without shared labels are significantly different at the 5% level

* A-B Mean values in each column without shared labels are significantly different at the 1% level

Both hybrid types displayed uniform feed consumption throughout the test, with no statistically significant differences observed in this parameter across the test periods. Notably, the average daily gain was statistically significantly higher ($p<0.01$) in broiler chickens of the Cobb 500 hybrid. On the other hand, the feed conversion ratio was significantly ($p<0.01$) inferior in chickens of the Ross 308 hybrid throughout all test periods. Despite non-statistically significant differences in mortality, broiler chickens of the Cobb 500 hybrid demonstrated higher vitality. Both hybrids maintained good health, with mortality rates below 3%. The European Poultry Efficiency Factor, serving as a comprehensive production indicator, was significantly higher ($p<0.05$) in all test periods for the Cobb 500 hybrid.

Upon comparing the production parameters of the fast-growing line hybrids Cobb-Vantress (2022) and Aviagen Ross 308 (2022), it becomes evident that the Cobb 500 hybrids exhibit a more prominent genetic potential throughout the entire examination period. Notably, these chickens had a lower initial body weight. However, due to lower mortality, improved feed conversion, and higher gains across all experiment phases, the Cobb 500 hybrid demonstrated higher values of the European Poultry Efficiency Factor. The European Poultry Efficiency Factor (EPEF) values showed an increasing trend until the age of 35 days in both hybrids. However, from days 1 to 42, the EPEF value decreased compared to the period from days 1 to 35. Razuki et al. (2011) also assert the preference for concluding the production phase in broilers before the 42^{nd} day of age. Sterling et al. (2006) and Lukić et al. (2022) determined significant differences in production results under the influence of genotype.

To provide a more comprehensive overview of the fundamental production parameters of broiler chickens, the data for each week are presented separately in Table 2. Feed consumption during the initial four weeks did not exhibit statistically significant differences between hybrids. However, significant distinctions emerged in the 5^{th} and 6^{th} weeks ($p<0.05$). Initially, Ross 308 broilers demonstrated superior consumption in the 5^{th} week, followed by Cobb 500 chickens in the 6th week. The average gain of broiler chickens in the first four weeks of the study was significantly higher in Cobb 500 ($p<0.01$). In the 5^{th} week, due to significantly higher feed consumption in the Ross 308 hybrid, there was also a significantly ($p<0.05$) higher gain in these chickens. However, the differences in gain in the 6^{th} week were not statistically significant ($p>0.05$). Throughout all tested weeks, the feed conversion ratio was less favorable in Ross 308 chickens, though the differences were not statistically significant in the 1^{st}, 2^{nd}, 4^{th}, and 6^{th} weeks. Analyzing the data for feed use efficiency in the 3rd and 5th weeks revealed statistically significant differences ($p<0.05$), indicating better conversion in Cobb 500 chickens.

Table 2. Production performance per test-weeks

Period	Hybrid	Consumption (g/day)		Gain (g/day)		Conversion (g/g)	
		x	Sd	x	Sd	x	Sd
1. week	Cobb 500	22.94	2.65	21.88^{A}	0.64	1.05	0.09
	Ross 308	22.78	1.01	19.37^{B}	0.29	1.18	0.08
	p-value	n.s.		<0.01		n.s.	
2. week	Cobb 500	60.71	1.61	45.48^{A}	0.08	1.34	0.04
	Ross 308	58.51	2.73	42.00^{B}	0.80	1.39	0.04
	p-value	n.s.		<0.01		n.s.	
3. week	Cobb 500	104.36	1.60	69.95^{A}	0.86	1.49^{b}	0.01
	Ross 308	104.18	4.70	65.33^{B}	0.36	1.59^{a}	0.06
	p-value	n.s.		<0.01		<0.05	
4. week	Cobb 500	150.74	0.83	101.24^{A}	1.07	1.49	0.01
	Ross 308	143.47	4.90	92.71^{B}	0.14	1.55	0.05
	p-value	n.s.		<0.01		n.s.	
5. week	Cobb 500	149.77^{b}	1.55	93.33^{b}	1.46	1.60^{b}	0.02
	Ross 308	164.01^{a}	6.42	97.00^{a}	1.36	1.69^{a}	0.04
	p-value	<0.05		<0.05		<0.05	
6. week	Cobb 500	183.39^{a}	2.87	91.48	2.78	2.01	0.09
	Ross 308	172.61^{b}	5.31	85.38	7.09	2.03	0.10
	p-value	<0.05		n.s.		n.s.	

x – average; Sd – standard deviation; n.s. – not significant

* a-b Mean values in each column without shared labels are significantly different at the 5% level

* A-B Mean values in each column without shared labels are significantly different at the 1% level

The average daily gain of Cobb 500 hybrid broiler chickens saw an increase from the study's commencement until the 4th week of age. Subsequently, after reaching 28 days of age, this parameter experienced a decline. In contrast, broiler chickens of the Ross 308 hybrid displayed more moderate gain values throughout the test. However, the increase persisted for an extended period, specifically until the 5th week of age, following which the average daily gain decreased in the final week. Consistent with our findings, Pascalau et al. (2017) and Tudorache et al. (2022) also observed notable differences in production outcomes influenced by genotype. A comparison of the production results from our test with those of Petričević et al. (2011) and Bjedov et al. (2011) underscores the evident genetic and overall progress of both hybrids over the past period. Considering the outcomes of the selection work in the last two decades and the results achieved in terms of broiler body weight and feed utilization efficiency, in line with the projections of Marcos and Fausto (2016), it is anticipated that fast-growing hybrids will attain an average body weight of 2.34 kg at 29 days of age within the next 10 years.

Table 3 displays the observed characteristics of carcass parameters. Although the pre-slaughter weight of chickens, the share of abdominal fat, and the circumference of chicken thighs were higher in Cobb 500 hybrid broiler chickens, the identified differences were not statistically significant. However, the carcass yield and chest angle, recognized as crucial indicators of conformation, were significantly lower in broiler chickens of the Ross 308 hybrid.

Table 3. Slaughter carcass traits of the hybrids

Hybrid	Sex	Salughter parameters									
		Pre-slaughter weight (g)		Yield (%)		Abdominal fat (%)		Chicken thigh circumference (mm)		Chest angle	
		x	Sd	x	Sd	x	Sd	x	Sd	x	Sd
Cobb 500	♂	3076.5	246.6	74.5	1.48	1.08	0.23	170.5	5.79	128.2	0.98
	♀	2652.2	120.5	74.7	0.79	1.19	0.58	154.8	6.28	129.2	1.33
	♂+♀	2864.4	288.7	74.6[a]	1.14	1.13[a]	0.42	162.7	10.00	128.6[a]	1.23
Ross 308	♂	3103.5	286.8	73.5	1.48	0.92	0.24	165.3	6.44	127.7	1.97
	♀	2462.5	250,0	73.8	0.76	0.95	0.37	154,0	10.75	123.2	4.62
	♂+♀	2783.0	421.7	73.6[b]	1.15	0.94[b]	0.3	159.7	10.32	125.4[b]	4.12
p-value		n.s.		<0.05		n.s.		n.s.		<0.05	

x – average; Sd – standard deviation; n.s. - not significant

* a-b Mean values in each column without shared labels are significantly different at the 5% level

The higher absolute values of chicken thigh circumference as an important characteristic of carcass conformation among the observed hybrids can be partially explained by the higher pre-slaughter body weight of the Cobb 500 broiler. Bearing in mind the fact that the increased content of abdominal fat is becoming an increasing problem in the production of chicken meat and that it has a high and positive correlation with the total amount of fat in the carcass, it is evident that broiler chickens of the Cobb 500 genotype had less favorable values compared to Ross 308. In addition, a higher proportion of abdominal fat in female chickens in both hybrids was observed. Significant differences in important slaughter parameters under the influence of genotype were confirmed in the research of Nikolova and Pavlovski (2009) and Abdullah et al. (2010). The obtained slaughter parameters are in accordance with the results of Hristakieva et al. (2014), who report a significantly higher yield in the Cobb 500 hybrid.

Conclusion

The production performances of both hybrids suggest the potential to achieve impressive results. The European Poultry Efficiency Factor values for both hybrids indicate that the broiler chicken production cycle should ideally conclude at 35 days. A comparative analysis of the two fast-growing (heavy-line) hybrids showed that the Cobb 500 broilers exhibited significantly superior production and slaughter performance.

In the conducted experiment, the Ross 308 hybrid broilers showed lower average gains, higher mortality, and a less favorable feed conversion ratio compared to the Cobb 500 broilers. Notably, the Cobb 500 broilers concluded the trial with a significantly higher relative yield of processed carcass and a more developed breast.

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ALTERATIONS IN THE PHYSICO-CHEMICAL COMPOSITION OF MILK ACROSS UDDER QUARTERS

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Abstract: Milk composition is fundamental in dairy production, afecting the health of cows and the quality of milk. This study explores alterations and mastitis effects on milk composition. Beyond somatic cell count, it analyzes fat, protein, lactose, and electrical conductivity across udder quarters. Comparisons among normal, subclinical, and clinical milk reveal significant differences in these parameters, emphasizing mastitis' adverse impact on milk quality. Continuous monitoring and prompt intervention are stressed. This research contributes valuable insights to dairy science, emphasizing the need for proactive measures in maintaining optimal dairy herd health and high-quality milk production.

Keywords: cow milk, udder quarters, qaulity, somatic cell count, milk composition

Introduction

Monitoring the health of dairy cows is crucial for ensuring the production of high-quality and hygienic milk (Boboš, 2012). In intensive milk production, mastitis is a prevalent and significant issue, impacting the productivity and profitability of dairy operations (Galfi, 2016). The presence of mastitis signals a substantial problem within the dairy herd, affecting milk yield, causing economic losses, and compromising the quality of raw milk.

Mastitis, characterized by inflammation of the mammary gland, can manifest as either clinical or subclinical. Clinical mastitis is easily identifiable through symptoms like redness, swelling, elevated temperature, pain, and decreased milk production. Subclinical mastitis requires a more thorough examination, involving the isolation and identification of inflammatory components in the milk (Nielen, 1995).

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Approximately 2-3% of dairy cows exhibit clinical mastitis, while a significant 40% may experience some form of mastitis (Stojanović, 2001). Addressing mastitis is crucial for the well-being of individual cows and the overall health and economic viability of the dairy operation. The findings presented by Forsback (2009) emphasize the importance of investigating variations in the composition of raw milk among individual udder quarters, particularly when there is an increased somatic cell count (SCC) at a low-to-moderate level within the overall composite SCC of cows.

The primary objective of this study was to investigate alterations in the physico-chemical composition of milk across individual udder quarters. In addition to standard analyses covering total protein, casein, fat, lactose, solids-non-fat (SNF), somatic cell count (SCC), and pH, we also measured the electrical conductivity (EC) of the milk. This comprehensive approach aims to deepen our understanding of how mastitis influences the overall composition of milk at the quarter level. Additionally, we monitored changes in milk composition based on the health status of the mammary gland.

Given the increasing consumer awareness and emphasis on quality and safety in products derived from healthy animals, there is a growing need for stringent quality control measures. Therefore, it has become essential to monitor milk quality directly on the farm to meet consumer expectations and ensure the production of safe and high-quality dairy products.

Materials and methods

Milk samples (N=69) were collected from a farm in the Pelagonian region utilizing a tied cow housing system (Trajkovska, 2023). Initial examination on a black pad was conducted to identify clinical mastitis or teat canal inflammation. Subsequently, California mastitis test (CMT) plates were used to detect subclinical mastitis. Two milk samples per quarter were obtained to determine somatic cell count (SCC/mL) and assess the conductivity and physicochemical parameters of the milk. The samples, transported in a hand-held refrigerator at a temperature of 5-8°C, reached the laboratory within 24 hours for testing. Results were categorized based on udder quarters (PL - front left, PD - front right, ZL – back left, and ZD – back right).

The California Mastitis Test (CMT) is based on the interaction of surfactants (alkylaryl sulfonate) with DNA polymer from leukocytes. This process induces DNA separation, causing the protein part to spontaneously transform into a gel. Result interpretation followed the methodology outlined by Galfi (2016).

Electrical conductivity (EC) was measured using a HANNA HI 98192 EC/TDS/NaCl/Resistivity conductometer with a range of 0-400 mS cm^{-1}. The analysis took place post-milking, maintaining sample temperatures between 20-25 °C. Approximately 50 ml of milk was utilized for the analysis.

The number of somatic cells was determined using the fluoro-opto-electronic method, employing the BENTLEY SOMACOUNT CC 150. The analysis followed standard MKC EN ISO 13366-2:2010 guidelines, involving bronopol preservation and preheating to 40 °C before assessment.

Parameters such as fat, protein, lactose, dry matter (SNF), density, casein, and pH were assessed using LactoScope FTIR Advanced.

Statistical significance among categories was evaluated at a significance level of 5% ($p<0.05$) and 1% ($p<0.01$) using the Student's t-test. The data were organized into tables, and analysis was conducted using Microsoft Office Excel and SPSS 20 statistical software.

Results and discussion

Table 1 presents alterations in the physico-chemical composition of quarter milk samples. The average somatic cell count for all cows exceeds 400,000 cells/ml per quarter, with counts for PD = 780.58 ± 757.03 cells ml^{-1}, PL = 799.32 ± 91.99 cells ml^{-1}, ZD = 628.712 ± 748.01 cells ml^{-1}, and ZL = 531.77 ± 566.39 cells ml^{-1}, indicating a concerning prevalence of mastitis on the farm. Notably, significant difference was observed among udder quarters in terms of somatic cell counts ($p<0.05$).

The percentage of lactose remains relatively stable across the quartets ($p>0.05$), with average values for PD = 4.5 ± 0.45%, PL = 4.47 ± 0.43%, ZD = 4.57 ± 0.46%, and ZL = 4.45 ± 0.57%. However, there are significant differences in electrical conductivity (EC) between PD (4.96 ± 0.89 mS cm^{-1}) and ZD (4.26 ± 0.77 mS cm^{-1}) ($p<0.05$). Minimal differences were observed, with values for PL at 4.64 ± 0.92 mS cm^{-1} and for ZL at 4.33 ± 1.04 mS cm^{-1}.

These results indicate a high prevalence of intramammary infection at the quarter level, highlighting the importance of implementing the California mastitis test on a daily basis on the farm (Trajkovska, 2023; Nakov, 2018).

Table 1.. Changes in the Physico-Chemical Composition of Milk by Quartets (N=69)

Udder quarters	Paramethers $\bar{x} \pm SD$							
	SCC/ml x 10^3	Fat (%)	Proteins (%)	Lactose (%)	SNF (%)	pH (%)	Casein (%)	EC (mS/cm)
PD (N=17)	780.58 ± 757.03a	2.07 ± 0.61	3.46 ± 0.35	4.55 ± 0.45	8.91 ± 0.57	6.82 ± 0.11	2.90 ± 0.27	4.96 ± 0.89a
PL (N=16)	799.32 ± 901.99	2.18 ± 0.60	3.57 ± 0.35	4.47 ± 0.43	8.94 ± 0.45	6.76 ± 0.12	2.97 ± 0.25	4.64 ± 0.92
ZD (N=18)	628.712 ± 748.01b	2.23 ± 0.67	3.56 ± 0.44	4.57 ± 0.46	9.05 ± 0.46	6.80 ± 0.10	2.98 ± 0.34	4.26 ± 0.77b
ZL (N=18)	531.77 ± 566.39b	2.14 ± 0.89	3.45 ± 0.26	4.45 ± 0.57	8.79 ± 0.62	6.82 ± 0.09	2.89 ± 0.21	4.33 ± 1.04

PL - front left, PD - front right, ZL – back left, and ZD – back right
*The presence of different letter in the same column indicates statistically significant differences at the level of a:b, with $p<0.05$.

Somatic cells in milk serve as a crucial indicator of udder health, reflecting the hygienic correctness and overall quality of the milk. The quantity of somatic cells in milk is influenced by a myriad of interconnected factors, including the lactation period, the number of lactations (i.e., age of the animals), milk yield, improper milking practices, stress, chronic diseases, and mechanical injuries to the udder tissue (Pyörälä, 2003; Trajkovska, 2023).

In Table 2, the alterations in the composition of raw milk during clinical and subclinical mastitis are presented, and a comparison is made with normal milk. Significant differences are evident between the normal milk group and the clinical and subclinical groups concerning electrical conductivity ($p<0.05$). Regarding the total somatic cell count, significant differences were noted between normal milk and the other two categories, which encompass samples with clinical and subclinical mastitis. Electrical conductivity (EC) in milk with clinical mastitis registers at 5.30 mS cm^{-1}, while in samples with subclinical mastitis, it is 4.80 mS cm^{-1}. No significant difference was observed between these two groups. For samples from cows with normal quarters, EC is 4.32 mS cm^{-1}. Similar findings were reported by Trajkovska (2023), where an increase in the number of somatic cells in milk (SCC ml^{-1}) corresponded to an elevation in electrical conductivity (EC). This aligns with the observed relationship between somatic cell count and electrical conductivity in the context of mastitis, emphasizing the consistent nature of this association across various studies.

The percentage representation of proteins, casein, and pH exhibits a consistent decrease across the three groups, and these changes are statistically significant at the $p<0.05$ level. Additionally, alterations were observed in the percentage of lactose, with the highest level observed in the normal group at 4.70%. As the inflammatory process occurs, the percentage of lactose decreases, reaching 4.39% in samples with subclinical mastitis and dropping further to the lowest value of 3.68% in samples with clinical mastitis ($p<0.05$). Similar findings were reported by Norberg (2004), where an increase in electrical conductivity among the three defined groups was observed. Notably, the values obtained for electrical conductivity in their study are higher; for instance, the values for normal milk are 5.30 mS cm^{-1}, whereas in our research, this value is indicative of clinical mastitis. Discrepancies in the results may be attributed to variations in the methodology of measuring electrical conductivity (flow measurement using sensors mounted on the milking line), the classification criteria, the duration of the research, and the number of examined samples.

Table 2: Changes in Milk Composition Based on Mastitis Status

Parameters	$\bar{x} \pm SD$ (CL) (N=9)	$\bar{x} \pm SD$ (SUB) (N=17)	$\bar{x} \pm SD$ (NL) (N=34)
SCC/ml x 10^3	1,725.54 ± 1,317.46a	787,622.04 ± 588.01a	394,794.73 ± 517.05b
EC (mS/cm)	5,30 ± 0,93a	4,80 ± 1,00a	4,32 ± 0,88b
Fat (%)	2,35 ± 0,40a	2,52 ± 0,69b	2,07 ± 0,73a
Proteins (%)	4,02 ± 0,29a	3,65 ± 0,28a	3,38 ± 0,35b
Lactose (%)	3,68 ± 0,59a	4,39 ± 0,40a	4,70 ± 0,32b
SNF (%)	8,47 ± 0,49a	8,93 ± 0,62b	9,02 ± 0,40b
pH	6,86 ± 0,10a	6,85 ± 0,11a	6,78 ± 0,09b
Casein (%)	3,30 ± 0,25a	3,05 ± 0,22a	2,85 ± 0,28b

*Values indicated by different letter in the same row exhibit statistically significant differences at the level of a:b, with $p<0.05$

Moreover, there is a significant decrease in lactose percentage in milk with subclinical mastitis (4.39%) and clinical mastitis (3.68%) compared to normal milk (4.70%). Ogola (2007) reported similar findings, noting an increase in Na, Cl, and pH, along with reduced lactose, Ca, and K percentages with a higher somatic cell count. These changes are linked to diminished secretory activity of cells and heightened mammary epithelium activity, as emphasized by Ogola (2007). This evidence underscores the intricate relationship between somatic cell count and various milk components, providing insights into the physiological changes during mastitis.

Conclusion

This study underscores the critical importance of monitoring udder health in dairy cows, especially regarding mastitis. Elevated somatic cell counts across all udder quarters indicate a significant prevalence of mastitis on the farm. Variations in electrical conductivity, particularly between front right and back right quarters, suggest potential intramammary infections. The comparison of normal, subclinical, and clinical milk underscores substantial differences in somatic cell count, electrical conductivity, and key physicochemical parameters, emphasizing mastitis's adverse impact on milk quality.

These findings stress the need for proactive monitoring practices, particularly the California mastitis test for early subclinical mastitis detection. Prompt mastitis management is crucial not only for individual cow health but also for overall economic viability. The observed changes in milk composition provide valuable insights for dairy farmers to implement effective quality control measures.

In light of growing consumer awareness and demand for safe dairy products, this study emphasizes aligning farm practices with these expectations.

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SULFUR-CONTAINING AMINO ACID SUPPLY IN ORGANIC POULTRY DIETS

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Abstract: The biggest challenge in organic poultry production is meeting requirements in sulfur-containing amino acids (SCAA), due to high demands first of all for methionine (Met), and the prohibited use of synthetic Met in diets, regarding organic farming rules. The poor quality of protein feedstuffs cannot always be compensated by increasing the concentration of dietary crude protein and by higher feed intake, because of disproportion and impaired utilization of amino acids. Incorporation in the diets of farm-produced feedstuffs and some of the alternative organic protein ingredients that are quality sources of SCAA may contribute to an increase in the level of Met.

Keywords: methionine, cysteine, layers, broilers, turkeys

Introduction

One of the main challenges in organic poultry production is meeting the nutrient requirements, particularly regarding essential amino acids (AA), (Adamović et al., 2005). Regulations considering organic poultry production do not allow the use of chemically extracted soybean meal and synthetic AA whereas rations must contain organically produced feedstuffs. The feedstuffs permitted for organic poultry production include protein sources rich in lysine (Lys) but relatively poor in sulfur-containing AA (SCAA).

The poor quality of protein feedstuffs cannot always be compensated by increasing the concentration of dietary protein and higher feed intake because of disproportion and impaired utilization of AA (Stojanović, 2020).

For poultry, especially laying hens and pullets, the SCAA – methionine (Met) and cystine (Cys) are regarded as the strongest limiting (NRC, 1994). Methionine is an essential AA important in feather growth, protein synthesis

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and breakdown, feed efficiency, and it impacts egg weight, rate of laying, and immune response. Poorer feed conversion, lower albumen and yolk weight, and reduced egg weight have been found to result from an inadequate supply of dietary Met for laying hens (Fanatico et al., 2018). Methionine deficiency also increases feather pecking and subsequent feather eating. For broilers, decreased feed intake, weight gain, feed efficiency, uniformity, and breast meat yield have been measured, as well as increases in fat deposition and a shift in thigh and wing yield when inadequate dietary Met is provided (Vieira et al., 2004). Similar negative impacts on body weight (BW), feed intake, and feed efficiency can be observed when turkey diets are deficient in Met.

Probably, one of the main problems in organic poultry nutrition is a ban on the use of synthetic Met (Hristov et al., 2006). To fulfill the Met requirements in organic poultry diets, levels of other AA need to be increased by 13-31% above optimal requirements due to using the greater levels of main organic protein sources (Van Krimpen et al., 2016). This results in increased harmful intestinal microbial growth, in causing a burden on the kidneys, increased manure moisture, poor litter quality, and increased NH_3 production, which leads to breast blisters and footpad lesions as well as increases in feed cost.

According to that Met and Cys are both SCAA, these two AA are typically considered together in feeds and poultry rations. Regardless of their relationship, due to the specific metabolic role of Met, there is a need for separate consideration of this essential AA. The required Met : Cys ratio ranges between 50 : 50 and 60 : 40, whereas in diets for broilers, the minimum concentration of Met relative to Cys is 52% (52 : 48 Met : Cys) (Pacheco et al., 2018).

This paper aims to provide an overview of the different aspects of the protein and SCAA supply in organic poultry production.

Sulfur-containing amino acids in organic poultry diets

Methionine is the most important source of organically bound sulfur in the organism and occurs in different proportions in poultry diet ingredients. For protein biosynthesis, the organism requires the essential AA to be provided in a specific ratio, and strong imbalances can lead to malfunctions, which may have a negative effect on feed intake and utilization, growth, egg production, and physiological parameters (Stojanović et al., 2006).

Table 1. The required content of methionine and methionine+cystine (mg/kg) in diets for laying hens for optimal egg number, weight, and egg mass (Waldroup and Hellwig, 1995)

Age, weeks	Egg number	Egg weight	Egg mass
	Methionine		
25-32	364	356	369
38-45	362	380	373
51-58	384	364	402
64-71	374	357	378
	Methionine+cystine		
25-32	608	610	617
38-45	619	636	627
51-58	680	621	691
64-71	690	601	676

For the layers 25-32 weeks, higher content of Met (0.38 *vs.* 0.23%) results in a 5.6% increase in egg size, while this improvement was 7.3%, 6.7%, and 6.0% at 38-44, 51-58, and 64-71 weeks, respectively (Waldroup and Hellwig, 1995).

Using three levels of CP – 19.3, 18.7, and 17.0% in diets for layers 18-41 weeks (Lohmann Silver and New Hampshire genotypes) that contained 0.39, 0.31, and 0.30% DM of Met, 0.85, 1.1, and 1.0% DM of Lys, and 0.74, 0.74, and 0.69% DM of Thr, with 11.3 MJ ME/kg, significantly affected production parameters like egg weight (57.3, 56.3, and 55.8 g), laying rate (82.0, 76.7, and 74.0%), and egg mass (47.4, 44.2, and 41.6 g/han/day). As foraging material, layers consumed maize silage and carrots, or alfalfa silage (Steenfeldt and Hammershøj, 2015).

Rose et al. (2004) reported that Hy-Line Brown layers fed an organic diet higher in CP (22.2%) and lower in energy (10.8 MJ ME/kg), with content of Met+Cys (7.0 g/kg) and Lys (8.5 g/kg) had somewhat higher egg output, but with a 17% higher feed intake, compared with hens fed conventional diet including synthetic AA (1.6 g/kg Met and 3 g/kg Lys) with 11.8 MJ ME/kg, with the significantly lower CP content (16.3%), and the same content of Met+Cys and Lys.

Pullets (Lohmann Tradition genotype) fed a basal organic diet supplemented with Met, compared to those on an organic diet, had heavier BW at 5 weeks of age, and this trend was maintained until 16 weeks. The pullets had access to the range from the 11. week of age (Acamovic et al., 2008). There were no treatment-associated differences in feed intake at any age, while the feed conversion efficiency was better for birds fed organic ration with added Met at 0-5 weeks.

Broiler genotypes that are used in organic farming show a growth pattern that is significantly different from that of conventional broilers. Therefore, their

AA requirements can be met by dietary CP contents that are lower than those from conventional diets. Diets with 20% CP content, 0.43% Met, and an ME concentration of 10.9 MJ ME/kg will provide acceptable growth rates in organically raised broilers (Peter et al., 1997). Carcass traits also are not affected by moderately reduced nutrient densities.

Table 2. Effects of dietary CP, methionine, and ME on body weight (BW) and feed conversion ratio (FCR) of "Label" broilers (Peter et al., 1997)

CP, %	Met, g/kg	BW (56 days), g	BW (84 days), g	FCR
15	3.2	1441	2525	2.74
17.5	3.8	1559	2683	2.69
20	4.3	1762	2780	2.74
22.5	4.9	1799	2791	2.70
25	5.4	1804	2786	2.75
ME, MJ/kg				
10.9		1745	2782	2.98
12.1		1662	2705	2.67
13.3		1636	2652	2.52

However, the CP and Met content of about 180 and 3.2 g/kg, are typical for organic broiler fattening diets, as well as 11 MJ ME/kg.

The increase of Met content (supplementation of synthetic Met) in an organic diet for dual-purpose type hybrid Barred Plymouth Rock × New Hampshire chickens 1-83 days of age was found to be effective and increased BW gain in the first period (1-49 days), while the value of feed conversion ratio was improved for the whole experimental period (1-83 days) (Koreleski and Świątkiewicz, 2009). Higher levels of Met increased breast meat yield and reduced abdominal fat content and also resulted in higher retention of N in the chicken body.

One of the determining factors in the composition of the carcass is the ratio of essential AA to the ME content in the diet, whereby the meat : fat ratio is improved by increasing proportions of AA (Huyghebaert et al., 1994). With reduced AA and sufficient energy content in the feed mixture, the feed intake increases to cover the AA requirement, while the energy excess is converted into fat. To prevent excessive fattening when providing a suboptimal supply of essential AA, the adjustment of the energy concentration is needed.

Lysine can be expected to be the first limiting amino acid for growing turkeys. The recommended values for the essential AA at 1-8 weeks (18.5, 6.7, and 12 g/kg of Lys, Met and Met+Cys, respectively for 0-4 weeks, and 16.1, 6.3, and

11.3 g/kg for 4-8 weeks) are unlikely to be reached with diets that comply with organic farming regulations (Zollitsch and Baumung, 2004). The AA undersupply of growing turkeys causes severe growth depression and highly variable body condition within the flock. In the case of young turkeys, the depression of feed intake caused by AA deficient diets may cause total feed refusal.

Possibility to meet requirements and sources of methionine in organic poultry

A practical option to ensure adequate content of SCAA in rations is to include available farm-grown feedstuffs and some other suitable protein sources characterized by particularly higher Met content.

High-Met corn hybrids are reported to contain approximately 50% more CP than conventional corn and 0.32 relative to 0.18% Met (Jacob et al., 2008). High-Met hybrids are also characterized by lower crop yields and they are more prone to fungal toxin development (aflatoxin), with possibly negative effects on poultry nutrition.

Naked oats have 8.5% more ME concentration than wheat and are comparable in ME to corn, but have greater CP (13%) and AA content, with 0.39% Met. Naked oats could to a larger extent replace corn, and partially soybean meal, in broiler diets (21-64 days), with incorporation into grower and finisher diets at levels of 18-72% (MacLean et al., 1997). The use of naked oats also improves feed conversion efficiency (egg mass/feed intake) in laying hens (Maunsell et al., 2004).

Pearl millet is higher in CP (12%), digestible Met (0.27%) and Cys (0.16%) than corn, but has a lower ME value, and could effectively replace up to 75% corn grain in the diets of laying hens without negative impact on egg quality or production (Mehri et al., 2010).

Heat-treated soybeans (36.2% CP, and digestible Met 0.43%, Cys 0.37%, and Lys 1.9%), as well as the soybean cake or expeller (43.9% CP, and digestible Met 0.55%, Cys 0.49%, and Lys 2.4%), are usually the main sources of protein in organic poultry diets due to their high nutritional value and protein digestibility, with the appropriate AA composition. However, like most legume proteins, the content of the SCAA compared to Lys is relatively low.

The grain legumes, other than soybean (peas, sweet lupin, faba bean), can be valuable protein sources (Table 3), but their use is limited by varying levels of anti-nutritional factors, such as tannins, lectins, protease inhibitors, and glycosides. An excessive inclusion of these feedstuffs in rations can lead to reduced feed intake. Some may be removed by thermal treatments, such as extruding, expanding, toasting, micronization, and pelleting (Stojanović et al., 2010). The CP digestibility of pulses is generally between 74-88%.

Table 3. Suitable protein and sulfur-containing amino acids sources in organic poultry diets

Feedstuffs	CP, %	Digestible or total* Met, %	Digestible or total* Cys, %	Recommended inclusion rate in diets
Canola expeller	31.5	0.55	0.57	Laying hens and broilers ≤ 5%;
Sunflower seed expeller, dehulled	33.5	0.68	0.39	Broilers ≤ 22%; may replace up to 50% of soybean meal in broiler and laying hen diets that are adequately supplied in Lys;
Sunflower seed	14.1	0.31*	0.24*	Broilers ≤ 5%; layers ≤ 10%; breeders ≤ 10%;
Peas	20.1	0.17	0.22	Broilers 25-30%; layers 15-20%;
Sweet lupin	30-36	0.17-0.21	0.37-0.44	Broilers ≤ 10%; layers ≤ 20%;
Faba bean	26	0.18	0.25	Turkeys up to the 35. day ≤ 10%; turkeys after the 35. day ≤ 15%;
Brewing yeast, dried	44.7	0.69*	0.46*	Broilers and laying hens 5-10%; may substitute up to 1/3 of the soybean meal in diets that are adequately supplied in Met and Lys;
Whole egg, spray-dried	45.1	1.5*	1.12*	Broilers and laying hens ≤ 10%;

Sainfoin seeds could be a highly palatable, protein source for poultry. Sainfoin seeds with hulls or dehulled contain 27.9% and 38.8% CP respectively.

Sesame seed expeller (45.1% CP) has lower digestible Lys (0.9%), but higher Met content (1.07%) than expeller-pressed soybean. Sesame seed expeller contains high levels of phytate and oxalates, and these compounds can reduce palatability and mineral digestibility.

Linseed expeller with 34% CP, 0.57% digestible Met, and 0.41% digestible Cys, also could be an alternative protein source for organic poultry diets.

Considering the protein feedstuffs that could be obtained on the market, the potato protein with 77-80% CP and respectable digestible AA composition

(1.65% Met and 5.45% of Lys) that is comparable with fish meal, certainly is one of the most valuable protein sources. It should be emphasized that potato protein concentrate is not usually available as a certified organic feedstuff, and is characterized by high price due to its use as an ingredient in human food production.

The corn gluten meal is also a significant source of digestible Met (1.29%) and Cys (0.81%), but it is low in Lys (0.81%), despite high CP content (60.4%). Corn gluten may complement soybeans due to a higher proportion of SCAA than soybeans with a lower Lys content. Corn gluten meal is relatively rich in xanthophyll pigments (up to 300 mg/kg), which are beneficial in enhancing egg yolk colour, and yellow skin for broilers.

Wheat germs are characterized by higher CP content (26.4%) relative to the whole kernel and could be a beneficial source of SCAA in organic poultry diets, with 0.39% digestible Met and 0.30% Cys, while the digestible Lys is higher (1.28%). Incorporation in poultry diets of corn germs expeller with 19,4% CP, 0.41% Met, and 0.43% Cys can contribute to an increase in the dietary level of SCAA.

Dried inedible egg products as by-products of the egg industry are an excellent source of Met, with levels of 3.1 and 1.5% in spray-dried egg white and whole egg, respectively. Burley (2012) reported that using spray-dried egg white and spray-dried egg blend at 7-9% of the diet to entirely replace synthetic Met in hen diets, improves egg quality and reduces feed intake. Egg products have to be sourced from organic layers to be used as feedstuff for organic poultry.

The considerably high levels of digestible Met are found in other products of animal origin, like casein (2.47%), and skimmed milk powder (0.96%, with digestible Lys of 2.75% and 35.6% CP), while whey powder is poorer in digestible Met concentration, approximately 0.19-0.37%. Casein, the solid residue from the coagulation of milk in cheese production, is high in CP (87.2%) with 84-99% digestibility, and also is high in digestible Lys (6.91%). Using high-protein sources such as casein, or milk powder can provide meeting the requirements in the essential AA for fattening turkeys (Bellof, 2002). However, it has a poor Met to Cys ratio (12.6 : 1 *vs.* optimal 60 : 40), and may not provide enough Cys to meet the poultry requirements. Casein has to be sourced from organic milk for use in organic poultry rations.

Access to a grass-clover pasture could substantially contribute to the protein supply of poultry. It is estimated that hens could obtain about 5-10% and broilers approximately 7% of their nutritional demands from foraging

(Hristov et al., 2006). When the mix of clover and grass is used, consumption of 10-30 g DM/hen/day is possible where there is no restriction to supplementary feeding, and this can rise to 20-40 g DM/hen/day with restricted supplementary feeding. The ME value of grass pasture for layers is approximately 6.4 MJ/kg DM (Stojanović et al., 2016). Access to pasture has a positive effect on behavior as it motivates the hens to spend more time foraging and less on feather pecking.

There are also could be found phytoadditives – herbal products as sources of Met. They may contain *Allium sativum*, *Allium cepa*, *Phaseolus mungo*, *Mucuna pruriens*, and some other plant species (Kalbande et al., 2009), and can be used as supplementary sources of Met at the rate of 1% in poultry diets.

Conclusion

The allowed use of only organic protein sources and prohibition of the use of synthetic AA, considerably make it more difficult to meet the requirements of organically raised poultry, primarily in digestible Met. High levels of CP must be used to meet Met requirements whereas the nutritional imbalances may lead to deteriorating production performances, product quality, animal health and welfare, and environmental impact, with increased feed costs. The farm-grown feedstuffs and alternative ingredients from organic sources with higher Met content may be used in diets for hens, broilers, and turkeys, to meet the nutritional requirements in this limiting AA.

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THE PHYSIOLOGICAL FORMS OF CALVES BEHAVIOUR IN THE ASSESSMENT OF WELFARE

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Abstract: The behaviour of calves is a response to stimuli from the environment causing movement, actions and changes in body position, affecting the health and welfare of the animals. We distinguish four categories of physiological behaviour:

- Behaviour related to basic needs such as movement, nutrition, rest and sleep.
- Exploratory behaviour that enables getting to know the living environment.
- Territorial behaviour and protection of safety and social order.
- Sexual and parental behaviour for the protection of the genome and the extension of the species.

The positive emotional state of animals is manifested through the freedom to express these forms of behaviour and social contact with other individuals, which reduces stress and fear. The goal of the care for animal welfare is to minimize exposure to negative emotions by optimizing these factors.
The present study, carried out on two farms with an intensive system of housing and rearing calves up to 30 days of age, showed significant weaknesses and deficiencies in the way calves are kept immediately after birth, up to 7 days of age. Namely, of 9 forms of physiological behaviour, 5 rated 1 or 2. The situation was somewhat better in the age category of calves between 8 and 30 days, where only one form, reproductive behaviour, was absolutely impossible.
In order to improve the quality of calf welfare, it is necessary to provide better rearing conditions that would enable the manifestation of basic physiological forms of behaviour.

Key words: calves, welfare, physiological forms of behaviour

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Introduction

The behaviour of calves is a conscious or unconscious response to stimuli from the environment, causing voluntary or involuntary movement, actions, changes in body position, etc. (Samolovac, 2016). Behaviour is of great importance for assessing the health and welfare of animals. All physiological forms of behaviour can be classified into four categories:

- behaviour of animals during movement, feeding, drinking, excretion, body care, rest and sleep,
- exploratory (research) behaviour of animals in order to familiarize themselves with the living environment,
- territoriality (marking and defence of territory) and behaviour of animals when establishing and protecting their own safety and social order,
- behaviour of animals for the purpose of protecting their own genome and prolonging the species (sexual and parental forms of behaviour).

All manifested activities and reactions of animals that arise as a response of animals to various stimuli from the environment or their organism can be classified into nine behavioural systems:

- behavioural system of reactivity,
- behavioural system of ingestion (taking of food and water into the body),
- behavioural system of research (exploratory) behaviour,
- kinetic behavioural system (movement),
- behavioural system of associations (social, collective behaviour),
- behavioural system of body hygiene,
- behavioural system of territoriality,
- behavioural system of reproduction,
- behavioural system of rest and sleep (Vučinić, 2006).

The positive emotional state of animals depends on their ability to freely express physiological forms of behaviour in every moment, with unhindered realization of social contact with other individuals. By optimizing these factors, the exposure of individuals to stress, fear and other negative emotions is reduced to a minimum, which is the goal of caring for animal welfare.

Material and method

The physiological behaviour of the calves was monitored in detail from birth to 30 days of age, on farms A and B, where dairy cattle of the Holstein-

Friesian breed were kept in the intensive rearing system. The sample on farm A consisted of 596 heads, while on farm B there were 572 heads. By analysing the technological procedures in the delivery area (0-7 days) and nursery (8-30) for the progeny, the degree of manifestation of physiological forms of behaviour was assessed, including reactive, feeding, kinetic, social, hygienic, reproductive, and behaviour related to rest and sleep of animals. The grades/scores ranged from 0 to 5, where higher scores indicated a greater degree of manifestation of a certain form of behaviour, while lower scores indicated a lack of opportunities for the manifestation of certain forms of behaviour. The manifestation of basic physiological forms of behaviour of calves, in the period from birth to 30 days of age, on farms A and B, largely depended on the established technology implemented on the farms, and partly differed depending on the observed category. The highest scores implied the highest degree of manifestation of a certain form of behaviour (5,4,3), and vice versa, the lowest indicated that there was no possibility to manifest certain forms of behaviour (1 - the condition is unsatisfactory, but there is a possibility to improve and 0 – bad condition with no possibility of improvement).

Results and Discussion

The results of the assessment of the possibility of displaying the physiological forms of behaviour of calves on farms A and B, at the age of 0 to 7 and 8 to 30 days, are presented in the following table.

Table 1: Assessment of the possibility of manifesting physiological forms of calf behaviour

Parameter/ Score	Age 0-7						Age 8-30					
	5	4	3	2	1	0	5	4	3	2	1	0
Reactivity – behavioural system			3					4				
Behavioural system of ingestion			3					4				
Behavioural system of research behaviour					1				3			
Behavioural movement system					1				3			
Behavioural system of social behaviour				2			5					

Behavioural system of body hygiene			3				5					
Behavioural system of territoriality				2				4				
Behavioural system of reproduction					1							0
Behavioural system of rest and sleep		4							3			

The degree of manifestation of the basic forms of physiological behaviour of the calves at the age of 0 to 7 days on the examined farms A and B was very similar and was scored similarly, considering that it is an identical way of keeping/rearing of calves. The best score was the ability to display eating behaviour (4), the lowest for the manifestation of reactivity, hygienic behaviour and sleep and rest (3), the score 2 for social behaviour and territoriality, while the lowest score was recorded for movement and reproductive behaviour. At the age of 8 to 30 days, the possibilities of manifesting the basic forms of physiological behaviour were more favourable. Calves were able to move, explore the environment, enter into social contacts with calves of the same or similar age, show their reactivity, feed without hindrance and maintain body hygiene, rest, which is indicated by scores 5, 4 and 3. Only the component of reproductive behaviour, maternal behaviour, was scored 0, which indicates that in the given technological conditions it was impossible to achieve any kind of contact between calves and mothers.

The behaviour of calves is a conscious or unconscious response to stimuli from the environment, causing voluntary or involuntary movement, actions, changes in body position, etc. Calves are exposed to stressful factors of the environment in different ways from birth, and one of the strongest is early separation from the mother. Therefore, it is necessary to provide them with conditions in which they can express all forms of their physiological behaviour in order to reduce the level of stress, fear, and other negative emotions to a minimum.

The biggest problem for calves age 0 to 7 days of life was the early separation from the mother and the immediate lack of maternal care. Calves are weaned within 1-2 hours after birth. According to several authors, this is one of the strongest stressogenic factors for both calves and mothers (*Budzynska and Weary, 2008; Stěhulova et al., 2008; Fröberg and Lidfors, 2009; Veissier et al., 2013*), which can be alleviated by enabling physical contact, e.g. by separating only with a fence (*Johnsen et al., 2015*). After being separated from their mothers, the calves are placed in an individual box on farm B, that is, they are tied to a bed in the maternity ward on farm A. In both cases, they are prevented from moving. However, on farms A and B, calves were able to hear their mothers and recognize them, which according to *Marchant et al. (2002)* is significantly for the preservation of welfare. Movement and exploratory behaviour were minimized due to the design of individual pens on farm B, and on farm A due to tethered calves, which is an even less favourable option (*Færevik et al., 2008*). Social contacts are limited to calves in adjacent pens or on adjacent beds. Calves in stalls (Farm B) could hear other calves, but not see them, while calves on beds (Farm A) could both see and hear more calves. Improvement of the condition can and must be sought in a different way of housing calves in the first 7 days of life.

In the nursery, calves aged from 8 to 30 days were placed in boxes for group housing. The possibilities of manifesting the basic forms of physiological behaviour at this age were more favourable, thanks to such accommodation. Calves were able to move, explore the environment, enter into social contacts with calves of the same or similar age, show their reactivity, feed and maintain body hygiene, and rest. Only reproductive, maternal behaviour was not possible.

Conclusion

The physiological behaviour of calves plays an important role in their development, health and welfare. Instincts with which animals are born, natural drives, such as searching for food, places to rest, movement, etc., play a key role in this form of calf behaviour.

The welfare of calves is an important aspect of farming practice, and providing optimal conditions for their physical and mental development is key to their health. This implies access to clean water and adequate nutrition, providing enough space for movement, space for rest and sleep, contact with individuals of the same species, similar or of the same age, etc. A high quality of calf welfare not only improves their standard of living, but also positively affects the quality and quantity of milk production later in life.

Work on improving the quality of life of individuals must be long-term and continuous, through the improvement of housing conditions, nutrition and care.

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REARING SYSTEMS AND PRODUCTION IN ORGANIC POULTRY FARMING

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Abstract: Poultry farming on free-range can be organized in various ways, depending on the type and characteristics of facilities and outdoor areas. Generally, these rearing systems can be categorized into a fixed facility free-range system and a mobile facility free-range system (structures on wheels). In organic poultry production, two organic production systems are prevalent: organic egg production and organic meat production.

Keywords: organic poultry production, organic meat, organic eggs.

Introduction

The production of a sufficient quantity of healthy food for the growing global population is one of the most crucial issues for the survival of modern society. One of the earliest predictions of a bleak future on this topic was made by Professor Jon Bedington when he proposed in his paper that the world's population would require a 50% increase in food production by the year 2030 (Bedington, 2009). The significance of food for individuals and society as a whole was explained by the renowned sociologist Abraham Maslow, the creator of the "hierarchy of needs." He emphasized that the need for food is a fundamental human requirement and that it is essential to satisfy this need before addressing all other needs (Maslow, 1954). Environmental conservation and intensive agriculture are not compatible, which is why organic farming has been recognized as an important solution to address these significant global issues.

With the realization of the negative effects of intensive industrial agricultural production as well as the consequences that consumption of products from the same can have from the aspect of consumer health (Laurence, 1991; Passille, 1997), interest in unconventional, especially organic, production is growing. All this has influenced that during the last decade, the volume of organic production on a global scale has increased significantly. Standards defined through laws and

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regulations that provide a framework for enabling good living conditions for domestic animals also contributed to the spread of organic production. These minimum standards, however, are not necessarily a guarantee for good conditions in terms of animal welfare and health (Sundrum, 2001; Kijstra and Eijck, 2006).

Organic agriculture is a management system that strives for ecologically and ethically acceptable, health-safe, socially just and economically profitable agricultural production (Petrović et al., 2020 and 2022). It is not only the production of high-value food and other agricultural products, but also implies a specific and high-quality relationship with natural resources, their use in meeting food needs, as well as the economic profitability and sustainability of production (Pavlović et al., 2011; Nikolić et al., 2013).

Rearing systems in organic poultry production

One of the fundamental characteristics of rearing systems in organic poultry production, whether it is organic egg or meat production, is the presence of outdoor access that primarily allows the natural behavior of poultry to be fully expressed. Poultry can move freely, enjoy fresh air and sunlight, and be reared for a sufficient duration, imparting a distinct quality to their products. On the other hand, having outdoor access is technologically more demanding compared to rearing poultry within buildings, mainly due to losses from diseases and predators. Additionally, outdoor production is seasonal because weather conditions significantly influence yield and product quality.

Rearing poultry with outdoor access can be organized in various ways, depending on the type and characteristics of facilities and outdoor areas. In general, these rearing systems can be categorized into:

- Fixed Facility Free-Range System
- Mobile Facility Free-Range System (Structures on Wheels).

These systems provide different approaches to organic poultry rearing, each with its advantages and challenges, contributing to the overall goal of sustainable and humane poultry production.

It implies an object of solid construction around which there is an outlet. The advantages are that you can build a solid building with water and electricity, so that you can also provide heating during the rearing of chickens. Poultry have access to the outlet located around the facility, and spend the night inside.

The disadvantage is that the poultry damages and destroys the vegetation on the outlet, so it is destroyed very quickly if rotation is not ensured, i.e.

moving the poultry from one part of the pasture to another. If an extraction system is not provided, the discharge turns into mud very quickly, especially in the area immediately around the facility, so the appearance both of dirty eggs and the risk of disease increases.

Providing access to open areas and allowing poultry to roam "outside" the enclosure of buildings allows them to express their natural behavior. They can freely roam, scratch, peck, dust bath, feed and supplement their diet on the outlet. Direct exposure to sunlight helps prevent disease. Overall, outdoor access contributes significantly to improving poultry welfare, but only if it is properly designed, has adequate space and is well maintained. Otherwise, it can present challenges for both birds and for production.

The outlet must be covered with vegetation. It is preferable to have trees inside the outlet or at least along its edges, which provide shade for the hens to avoid excessive sunlight. The soil must have sufficient drainage to prevent water retention and mud build-up. Food and water must be provided to poultry outdoors, reducing the need for constant entry into facilities.

Feeders should be covered from above to prevent wild or flying birds from reaching them. Covering the feeders also protects them from the rain. The water must always be clean and fresh. Both feeders and waterers should be mobile, allowing them to move around the open space.

If the outlets are without vegetation and trees, i.e. represent a "cleared space", it is necessary to build canopies at the outlet as well, because they provide protection to birds from predators and weather conditions - primarily sun and rain. Without them, the poultry would feel uncomfortable because they do not like to be exposed to flying predators. Also, birds do not like strong sun and wind, so some protection is necessary for them. Some researches have shown that the poultry has no problem to "pluck" and move around in the open space, but prefers to rest in some bushes or among the branches.

Research in Great Britain has shown that in large flocks reared in the "free range" system, only 15% of the birds from the flock are outside, while the rest are inside the facility. If the outlet was better covered by trees, the number of poultry at the outlet is higher. The use of the outlet depends on the time of year (in the spring it is used more than in the winter), as well as on the time of day (the hens leave the least at noon). In order to motivate the poultry to move away from the buildings and use the entire outlet, it is necessary to place the feeders and shelters a little further from the building. Some birds that are extremely active move even 100 m away from the building.

Since birds are reluctant to leave the facility, it is necessary to encourage their exit and movement, and this can be achieved by building and designing the facility. The buildings should be small, and the openings for the exit of the poultry should be relatively large and wide. There should be a sufficient number of openings for exiting the building (4 m per 100m^2) so that the poultry do not pile up on the openings and that several of them could enter and leave the building at once. This is very important with outlets, because when the birds sense danger, they must be able to quickly fly into the facility and seek protection, without the risk of getting stuck.

Another factor that depends on the degree of use of the outlet is the reared genotype. Broiler chickens, which are not usually used in organic production, are much heavier and reluctant to move. Much more active are slow-growing hybrids for meat production, light hybrids for egg production and combined breeds of chickens. During the day, they like to go out to bars and rummage in search of food, and at night they enter buildings and spend the night there. They mostly do this instinctively, although some chickens have to be taught to enter the facility on their own at night.

Part of the nutrients are taken in by the birds through grazing, and part by digesting insects and earthworms, thus obtaining high-quality animal proteins. They can use the plant part relatively poorly because chickens, although they have two caecums, do not digest cellulose (5-8%), so it is basically difficult to estimate how many nutrients are actually taken into the body on pasture. According to some estimates, pastures can replace 5-10% of the feed, but most experts advise not to count the contribution of pastures to the diet, but to balance nutrients as if complete needs are met by food.

The maintenance of the outlet is a key moment in the system of organic production of meat and eggs on the outlet. The vegetation must be maintained, and it is preferable that the plants are young, in the early phase of the vegetation, because otherwise the poultry will not consume them, they will just trample on them. If the grass is too tall, the poultry make tunnels through the tall grass and pass through them just to get to the feeder, and the rest of the outlet is not used at all. Tall grass, on the other hand, is often wet, so it is suitable for the development of parasites. The moisture from the grass will also wet the feathers of the poultry, so they will bring that moisture into the building, which is not good because it will stain the mat.

Outlet rotation allows the pasture to rest, the vegetation to recover from trampling, and to prevent the build-up of manure material and the development of pathogens. Outlet should be rotated for a minimum of two to three months, although a month would be best. With broilers, the outlet can be rotated after each turn (after the birds are sold and delivered), but it cannot remain on the same outlet during the entire production period. Rotation is most easily done by dividing the outlet into 4 parts, so the birds move from one part to another after a while. In America, the Soil Association recommends that the outlet be rested for 12 months between flocks, with a minimum of 9 months. This means that if the outlet is divided into 4 parts, the poultry can stay in each part for 3 months, and then move to another part. In this way, each part is in use for 3 months and rests for 9 months.

The problem with non-rotating outlet is that the vegetation is completely destroyed, and the substrate turns into mud and dirt. This is introduced into buildings and nests and the eggs are contaminated. Also, pathogenic microorganisms multiply on such a outlet, so the risk of disease increases. If less than 50 hens are reared, outlet rotation is usually not necessary.

The system with movable objects on the outlet enables their movement, which enables the even use of the entire grass area and the preservation of the outlet. Mobile homes always have a smaller capacity because they have to be moved regularly. They can be quite simple - in the form of a canopy with a wooden structure or a very solid closed house. They are mostly on wheels, so they can be moved with the help of a tractor or an all-terrain vehicle. Moving is done every few days depending on the plan of using the outlet. The houses must be moved at least once a week because after that the plants can be permanently destroyed. It is estimated that it takes about a month for the outlet to recover, but it primarily depends on the climatic conditions. It is important to make a good shift plan so that one part of the pasture is used and the other parts are rested and regenerated.

If the houses are not on wheels, it is more difficult to move them, although they can also lean and move to another part of the pasture. Those houses should be of very light construction, more reminiscent of canopies, so that they can be easily moved to another part of the outlet.

Rearing systems in organic poultry production

There are two systems of organic poultry production:

- ***organic egg production***
- ***organic meat production.***

Unlike conventional production, these two systems are not clearly separated, but are usually integral and complement each other.

In the production of organic eggs, it is similar to the free range system, with the fact that the producers must comply with the Rulebook of Control and Certification of Organic Production and methods of Organic Production (2021), according to which food must also come from organic production, with the prohibition of the use of additives, colors and medicines. In essence, the system for organic egg production is regulated by law with restrictions related to the capacity and technological norms in the construction of facilities and, above all, to the nutrition and treatment of laying hens. Poultry farms that produce eggs in this way are specially registered, regularly controlled by the inspection of the competent state authority and must keep the prescribed records. During egg production in an organic system, layers should have constant access during the day to an open outlet that is covered with vegetation and whose maximum population is 580 layers per hectare, i.e. minimum 4 m^2 per hen (Rulebook of Control and Certification of Organic Production and methods of Organic Production, 2021). Feeders and drinkers are placed at the outlet.

In organic production, laying eggs usually remain in production for a second year, after the moulting period. In the second year, the laying capacity is lower, but larger eggs were layed. Namely, the production of organic eggs usually starts at the age of the flock between 12 and 14 weeks of age. Until that age, the flock was reared under similar conditions as in conventional (intensive) production. From the 14th to the 20th week, there is a preparatory period, and at 20 weeks of age, laying hens start producing eggs (Lampkin, 1997). The maximum production rate (95%) is achieved at 28 weeks of age, while production intensity until the end of the production cycle (up to the 72nd week of age, i.e. 52 weeks of egg production) ranges between 60% and 65%.

From a nutritional perspective, it is ideal to produce as much of the total feed requirement as possible on one's own farm. This represents the most favorable solution in terms of reducing the cost of purchasing animal feed and is harmonized with the dominant characteristic of organic poultry production. Some calculations show that if all the nutrients in the diet are produced on the farm, with 1 hectare of arable land, the needs of about 100 laying hens can be satisfy (Hörning, 1995). Basically, the nutrition of laying hens is organized in such a way that they are provided with a complete mixture of food in the barn, and additional nutrition (grains, semolina, green fodder) is provided in the open area. External access allows poultry access to additional food sources in addition to the food provided by the farmer (Gordon and Charles, 2002).

In an organic meat production system, broilers are usually kept indoors on litter until 28 days of age (maximum 11 chickens/m^2) and fed standard diets with 21.5 to 23.0% crude protein, i.e. 3,050 to 3,080 kcalME/ kg. After this age until the end of the growing period (minimum 81 days), chickens should have access to grassy open areas (minimum 4 m^2/chicken), and different grains can be used, primarily corn, oats, wheat and certain varieties of triticale. serve as the main food. Poultry in open spaces also benefit from supplementary feed such as green feed, seeds, insects, worms, etc. This approach not only provides a significant amount of high-quality protein, but also provides the necessary amounts of vitamins and minerals, which significantly improves the quality of the meat. Van de Weerd et al. (2009) point out that outdoor access stimulates locomotion, leading to stronger leg muscles, yellower meat, higher proportion of breast and thigh meat and lower abdominal fat content in organically raeared broilers. In addition, a better sensory quality of the breast muscle was observe.

Conclusion

Growing awareness of ecosystem disruptions and the overall threat to biodiversity has required a re-examination of the technologies and methods used to accelerate production growth in all human activities, including agriculture. Despite the fact that a large number of individuals of different types of domestic animals are bred all over the world and in our country, there is an increasing objective danger of their rapid disappearance due to the decline of adaptive abilities.

The advantage of organic production of meat and eggs in poultry farming is that this kind of production enables mitigating the negative effects of social development on the ecosphere and the human population as a whole. Organic production of meat and eggs can cover all the above-mentioned requirements regarding the preservation and well-being of the environment in the rearing of different types and categories of poultry, with realistic possibilities of development in our conditions. It is necessary to mention that one of the most significant characteristics of organic poultry farming is the unity of plant and animal production.

Based on the above, it can be concluded that areas with natural potential, opportunities for sustainable development and environmental protection should be sought as development opportunities for underdeveloped countries. For Serbia, the concept of organic poultry farming represents just such an opportunity.

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THE EFFECT OF PROTEASE ENZYME ON THE MEAT CLASSES OF TWO BROILER CHICKEN HYBRIDS

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Abstract: The aim of the study was to evaluate the effect of three protein levels (with or without protease enzymes) on the weight and percentage yield of each meat class in two genotypes of broiler chickens (fast-growing hybrid Cobb 500 and medium-growing hybrid Master Gris). Each genotype was divided into a control group (C group) fed with a commercial feed mixture and an experimental group fed with a low protein diet of 4% (E-I group) or 6% (E-II group) in relation to the control group, with the addition of 200 mg/kg Ronozyme ProAct (E-I group) or 300mg/kg Ronozyme ProAct (E-II group).

Weight and percentage yield of the meat classes were influenced by the genotype. The fast-growing hybrid Cobb 500 had higher masses of all meat classes and a higher percentage of class I meat, while the percentage of class II and III meat was lower than in the medium-growing hybrid Master Gris ($P<0.05$). Feeding had no effect on the weight and percentage yield of the individual meat classes in either hybrid ($P>0.05$).

Keywords: hybrids of chickens, protease enzyme, meat classes.

Introduction

The quality of poultry meat is the result of complex interactions between genotype, age and sex of the birds and the managment system (Uhlířová et al., 2018). It is well known that nutrition is one of the key factors influencing the quantitative and qualitative characteristics of poultry meat and the profitability of production.

Nowadays, soybeans are the main source of protein for broiler diets (Tavaniello et al., 2022). However, soybean meal contains anti-nutritional factors such as trypsin inhibitors that reduce nutrient availability and limit the amount of soybean meal that can be included in animal feed (Park et al., 2020). The addition

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of enzymes to broiler feed is known for its economic, environmental and nutritional benefits (Jabbar et al., 2021). The addition of exogenous protease improves growth and increases the digestibility of crude protein and metabolizable energy in broiler diets while improving the utilization of amino acids as it cleaves antinutritive factors such as trypsin inhibitor (Ndazigaruye et al., 2019).

Medium-growing broiler genotypes are more resistant to heat stress compared to conventional fast-growing hybrids (Pietrzak et al., 2020), and their meat is also interesting on the market (Devatkal et al., 2019). Research on the use of protease enzymes in feed for broiler chickens has mainly been conducted on fast-growing genotypes (Dosković et al., 2023b; Hafeez et al., 2021; Jabbar et al., 2021; Park et al., 2020;…). However, limited information is available on the effects of different protein levels using protease enzymes on the meat quality of medium-growing chickens kept in an intensive rearing system. The aim of the study was therefore to evaluate the effects of three protein levels (with or without protease enzymes) on the meat quality of fast- and medium-growing chickens, including the interactions between the two factors.

Materials and methods

Two genotypes were used for the experiment: the fast-growing hybrid Cobb 500 and the medium-growing hybrid Master Gris. Each genotype was divided into a control group (100 chickens, group C), which was fed a standard commercial feed mixture, and an experimental group, which received 4 % (100 chickens, group E-I) or 6 % (100 chickens, group E-II) low-protein feed with the addition of 200 mg/kg Ronozyme ProAct (group E-I) or 300 mg/kg Ronozyme ProAct (group E-II) in relation to the control group. The control groups received mixtures with 22% crude protein in the starter phase (0 - 21 days), 19% crude protein in the grower phase (22 - 35 days) and 17% crude protein in the finisher phase (36 - 63 days).

All birds were reared under environmental conditions that meet the requirements for broilers. Feed and water were available to the chickens *ad libitum*.

At the end of the experiment, at 63 days of age, 20 chickens per group (sex ratio 1:1) were selected and slaughtered for meat analysis.

After the carcasses had cooled, they were cut into their basic components: breast, drumsticks, thighs, wings, back and pelvis. These primal cuts were divided into meat class I (breast, drumsticks and thighs), meat class II (wings) and meat class III (back and plevis). These meat classes were measured and, based on the weight of the meat class and the weight of the ready-to-grill carcass, the proportions of the meat class in the dressed carcass were calculated.

The results were analyzed by Stat Soft Inc Statistica For Windows (Version 7.0., 2006) program. Two-factor (diet treatments and hybrids) analysis of variance and LSD test to compare the treatment means was applied ($P<0.05$).

Results and discussion

The results of the weight of certain classes of meat are presented in Table 1.

Table 1. Weight of different classes of chicken meat on the 63th day of fattening, g

Treatment			Class I (breast, thighs, drumsticks)	Class II (wings)	Class III (back, pelvis)
Hybrids	Groups				
Cobb 500	C	$\bar{X}$	1874.90^{a}	297.39^{ab}	643.74^{a}
		Sd	276.86	46.03	74.74
	E-I	$\bar{X}$	1880.37^{a}	302.24^{a}	642.00^{a}
		Sd	266.21	36.30	80.70
	E-II	$\bar{X}$	1798.79^{a}	297.79^{ab}	625.43^{a}
		Sd	203.18	34.49	71.41
Master Gris	C	$\bar{X}$	1493.27^{b}	277.22^{b}	576.76^{b}
		Sd	197.58	41.05	73.88
	E-I	$\bar{X}$	1454.60^{b}	276.87^{b}	570.66^{b}
		Sd	180.93	35.27	71.88
	E-II	$\bar{X}$	1437.49^{b}	274.41^{b}	550.73^{b}
		Sd	182.71	35.93	67.13

$\bar{X}$-Average, Sd - Standard deviation

Different superscripts (a, b) indicate a significant differences between groups ($P<0.05$)

From the data analysis, it can be concluded that there are differences in the weight of certain meat classes between the studied genotypes, so that the fast-growing Cobb 500 chickens had a higher weight in all meat classes than the medium-sized Master Gris chickens ($P<0.05$). The chickens from the feed treatments of both hybrids had similar weights in the meat classes, from which we conclude that the formulations used in the complete mixtures for broilers had no effect on the weight of the different meat classes ($P>0.05$). The fact that the reduced crude protein content with or without the enzyme protease has no effect on the composition of the carcass is consistent with the observations of Dosković et al. (2023a), Chodová et al. (2021), Ndazigaruye et al. (2019).

The percentages of certain chicken meat classes in the dressed carcass of slaughtered chickens are shown in Table 2.

Table 2. The percentage of the different chicken meat classes in the dressed carcass on 63 day of fattening, %

Treatment			Class I (breast, thighs, drumsticks)	Class II (wings)	Class III (back, pelvis)
Hybrids	Groups				
Cobb 500	C	$\bar{X}$	63.80^{a}	10.14^{b}	22.03^{b}
		Sd	2.42	0.89	1.50
	E-I	$\bar{X}$	64.07^{a}	10.35^{b}	21.94^{b}
		Sd	2.26	0.71	1.23
	E-II	$\bar{X}$	63.40^{a}	10.51^{b}	22.06^{b}
		Sd	1.87	0.80	1.20
Master Gris	C	$\bar{X}$	60.97^{b}	11.30^{a}	23.57^{a}
		Sd	0.97	0.55	0.73
	E-I	$\bar{X}$	60.46^{b}	11.51^{a}	23.73^{a}
		Sd	0.98	0.53	0.75
	E-II	$\bar{X}$	60.76^{b}	11.60^{a}	23.30^{a}
		Sd	1.19	0.46	0.83

$\bar{X}$ - Average, Sd - Standard deviation
Different superscripts (a, b) indicate a significant difference between groups (P<0.05)

The genotype had a significant effect on the investigated carcass traits (P<0.05). Compared to Cobb 500 chickens, Master Gris chickens had a lower proportion of breast, drumstics and thighs, i.e. class I meat, and a slightly higher proportion of wings (class II meat) and back and plevis (class III meat) (P<0.05). Kreuzer et al. (2020) state that the genotype has the greatest influence on the composition of the carcass, while Chodová et al. (2021) point out that fast-growing chickens have the highest proportion of breast (P<0.001), while medium-growing chickens are in the middle between fast and slow-growing genotypes.

The addition of exogenous protease to the feed at a concentration of 200 mg/kg (E-I group) or 300 mg/kg feed (E-II group) with a reduction in crude protein content of 4 or 6 % compared to the control feed (C group) had no significant effect on the proportion of all meat classes (P>0.05). There were also no significant interactions observed between the diet and the genotype on the percentage of all meat classes (P>0.05). Duque-Ramírez et al. (2023) also found that there were no significant differences in the inclusion of proteases in the diet (P>0.05) in relation to the partial yield of carcass cuts of meat classes I and II 42 days of fattening. Dosković et al. (2023a) found that only in female chickens were there differences in the percentage yield of class I and III meat between the E-I (200mg Ronozyme ProAct/kg feed) and E-II (300mg Ronozyme ProAct/kg feed) feeding treatments ($P<0.05$) in chickens aged 49 days, while there were no differences between the standard feeding treatment (C group) and the E-I group or E-II group in the carcass quality parameters examined ($P<0.05$).

Conclusion

The present study focused on the comparison of carcass quality parameters such as mass and percentage of each meat class of medium growth genotypes - Master Gris (reared in an intensive feeding system, characteristic of commercial fast growing chickens) with the hybrid Cobb 500. In addition, the application of protease enzymes with the reduction of crude protein content in two hybrids with different growth intensity and their effect on these chicken carcass quality parameters was analyzed.

The results showed that the genotypes studied differed in weight and percentage yields of the meat classes. The fast-growing hybrid Cobb 500 had higher masses of all meat classes and a higher percentage of class I meat, while the percentage of class II and III meat was lower than in the medium-growing hybrid Master Gris ($P<0.05$). For both hybrids, the feed had no significant effect on the weight and percentage yield of the individual meat classes ($P>0.05$). The proposed feed formulations for broilers, which provided for a 4% reduction in crude protein content, i.e. 6% at each fattening stage (starter, grower, finisher) with the addition of 200 mg/kg feed or 300 mg/kg feed protease enzymes (Ronozyme ProAct) compared to the standard feed, did not cause any changes in the weight and percentage yield of the meat classes ($P>0.05$).

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REGULATORY PERSPECTIVES ON NITRATE USAGE IN ORGANIC AGRICULTURE: A FOCUS ON EU LEGISLATION

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Abstract: This manuscript explores the critical issue of sustainable agriculture and responsible land use, emphasizing the significant role of good agricultural practice (GAP). Despite land being a fundamental resource, a considerable number of farmers, including those in livestock farming, often exhibit irresponsible behavior towards its preservation. Recognizing this as a fundamental concern, the European Union has implemented stringent regulations to address the issue. The initial section of the paper outlines the identification of the problem and provides a concise overview of the European Union's initiatives in this realm. The subsequent section delves into the contentious use of nitrates, a prevalent element in fertilizers aimed at boosting agricultural production. The authors present fundamental aspects of the code of good agricultural practice and its overarching implications, with a focus on the potential application of the code and the integration of the nitrate directive into the legislation of the Republic of Serbia. The manuscript concludes by highlighting the current environmental state in agriculture, asserting that it falls short of satisfactory levels. The authors emphasize the necessity for additional measures to align with the accepted standards of the European Union, underscoring the ongoing imperative for enhanced environmental stewardship in agricultural practices.

Keywords: Nitrate Directive, EU Commission, good agricultural practice, environment, nitrate

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Introduction

In addition to industry and urban areas, agriculture can cause very negative consequences and various disturbances in the environment. That is why in the EU, special care is taken to protect the environment, that is, the pressure on the environment that comes from agriculture.

The intensive development of a country and its economy, including agriculture, is almost as a rule accompanied by an increase in pressure on the environment and a consequent decrease in the quality of basic natural resources, i.e. water, air and soil. Therefore, the strategic determination of many countries in recent decades has changed in the sense that they build their development on the principles of sustainable development - harmonized and coordinated economic development with environmental, social and other policies. The member states of the European Union are no exception. On the contrary, environmental protection is one of the goals that is incorporated in the first of the three pillars on which the European Union rests. This practically means that the number of regulations regulating this area in the EU increases significantly from year to year.

The main instrument used to regulate and develop agriculture in the EU for more than 40 years is the EU's Common Agriculture Policy (CAP, 2023 - 2027). The latest CAP reforms continue to implement a series of radical changes that have a significant impact on all EU farmers. EU policy, as well as common agricultural policy, increasingly aims to reduce the risk of environmental degradation and encourage farmers to continue to play a positive role in maintaining the landscape and preserving the environment. Therefore, the strategy of the current Common Agrarian Policy is largely focused on the sustainability of the agro-eco system.

Measures integrating environmental protection into the CAP include environmental protection requirements that farmers must fulfill on their farms to receive subsidies (cross-compliance), incentives integrated into market and income policies, and targeted environmental protection measures which are part of the rural development program (or agriculture-environment schemes).

During the development of agriculture, the highest degree of industrialization was achieved in animal husbandry. However, with the industrialization of animal husbandry, the number of animals on farms grew to a level that made it impossible to adequately store and apply manure on the available agricultural land, which is why the pressure on the environment and its pollution also increased. First of all, it refers to excess nitrates and their leaching (leaching) into surface and underground waters.

As a result, in some EU countries, a limit on the number of animals per unit of area has been introduced, which is expressed as the number of conditional herds or groups per unit of area. Therefore, the maximum number of livestock that an agricultural farm can raise is conditioned by the land at its disposal. Also, it is mandatory to dispose of manure in appropriate warehouses to store the minimum capacity for a six-month production of manure on the farm. All these and many other rules for farm management are unified in the Code of Good Agricultural Practice, which each EU country must develop according to its specificities.

Nitrate Directive

Intensive use of artificial fertilizers, uncontrolled use and discharge of manure produced on farms and changes in land use are the main factors behind the progressive increase of nitrates and nitrites in European waters over the last 25 years. In the compilation *The European Nitrogen Assessment: Sources, Effects and Policy Perspectives*, in which the works of experts from 21 European countries and as many as 89 organizations were published, an assessment was published according to which the annual damage caused by nitrates at the level of the European Union is estimated at 70 and 320 billion euros, that is, from 150 to 750 euros per inhabitant (Brink *et al.*, 2011). Loss of soil fertility is one of the major problems of conventional agriculture, since fertility is depleted, which on the other hand is insufficiently replaced by artificial fertilizers (Kelam, 2016).

With a special Directive, the European Union limited nitrate pollution originating from agricultural pollutants. It is one of the first regulations by which the EU regulates the problem of environmental protection in agricultural production and which resulted in significant changes in agriculture.

The so-called EU Nitrate Directive obliges member countries to identify waters threatened by nitrate pollution and those that may be threatened in the near future. The criterion is that the concentration of nitrates should be below 50 mg/l in fresh waters and that eutrophication should be prevented. Agricultural regions that contribute to such pollution in identified polluted/potentially polluted waters must be designated as endangered zones. In such zones, each country should adopt and apply special measures, ie action programs and monitoring programs, with the aim of reducing the concentration of nitrates below the prescribed values.

The Nitrates Directive also obliges member countries to establish a national Code and (rules) of good agricultural practice, which should contain provisions covering at least those items listed in Annex II A. This Code will be applied by farmers on a voluntary basis throughout the country, and in endangered/sensitive zones its application is mandatory.

This directive stipulates, among other things, that certain measures are to be applied in endangered zones to ensure that, on livestock farms, the amount of manure applied to the fields each year will not exceed the amount of manure containing 170 kg N/ha. Some EU countries apply this restriction throughout their territory.

The Nitrate Directive is practically the best known for the introduction of the limitation of manure application to 170 kg N/ha/year and the obligation to establish a national Code (Rule) of good agricultural practice in EU countries.

Codes of Good Agricultural Practice in Accordance with EU Regulations

EU member states must define standards of good farming practice that can be verified at regional and national level. The complexity of the relationship between agriculture and the environment (harmful and beneficial processes, diversity of local conditions and production systems) made it necessary to integrate environmental protection into the CAP context (CAP, 2023 - 27). Central to understanding this relationship is the principle of good farm practice that corresponds to the type of agriculture that a conscientious farmer will follow in a given region. This includes as a minimum compliance with EU environmental protection laws and national laws. Good farming practice includes, among other things, compliance with the requirements of the Nitrates Directive.

In relation to rural development policy, compliance with minimum environmental protection standards is a condition for obtaining financial support for several different rural development measures, such as aid for investments in agricultural facilities, assistance to young farmers and improvement of processing and marketing of agricultural products. Support to less developed areas also requires compliance with the rules of good agricultural practice.

The Code of Good Agricultural Practice constitutes minimum standards for farm management and these include natural resource protection, environmental management, workforce safety, animal health and welfare, food and feed safety and health protection.

Although it does not directly regulate agriculture, the Water Framework Directive is also very important for the implementation of the principles of good agricultural practice (Directive 2000/60/EC), which represents the umbrella law of all EU regulations related to water. This directive requires the identification of any source of significant pressure that, by itself or in combination with other pressures, may lead to endangering the status of good water. If point or diffuse sources from agriculture are identified as significant burdens, all watercourses with a risk of not fulfilling the status of good waters due to environmental influences must be identified. In this sense, the agricultural practice must be changed in such a way that all water courses get the status of "good water".

The directive concerning the prevention of pollution should also be mentioned (Council Directive 96/61/EC), repeatedly revised and changed (Directive 2008/1/EC, Directive 2010/75/EU), and which directive prescribes the mandatory application of the best available techniques in order to reduce harmful effects from the industry (BAT).

In general, several EU regulations mandate the adoption of rules, conditions or standards in agriculture related to environmental protection, which farmers must comply with. All those related terms appearing in the EU regulations are designated as common Standards of Good Farming Practice (Regulation (EU) No 1305/2013). In this sense, it is worth mentioning the Statutory Conditions of Management (SMR) (Regulation (EU) No 1307/2013) which represent a set of 18 EU directives in the field of nature, agricultural environment, food safety and animal welfare, Good Conditions for Agriculture and the Environment (GAEC) (Regulation (EU) No 1307/2013) which represent regionally determined measures that farmers must comply with in relation to minimum standards for soil cultivation and Best Available Techniques (BAT) (Directive 2010/75/EU) described as „the most effective and advanced stage in the development of activities and their methods of operation that indicate the practical suitability of certain techniques for determining the basic values of pollution limits, which are designed to prevent and, where this is not feasible, reduce pollution and the impact on the environment as a whole.“

Current situation in the Republic of Serbia and the Possibility of Implementing the Nitrate Directive

After signing the agreement of the Republic of Serbia on cooperation and association with the European Union (The Law on the Confirmation of the

Agreement on Stabilization and Association between the European Communities and their member states, on the one side, and the Republic of Serbia, on the other, 2008), our legislation will have to incorporate and implement all EU regulations in the process of harmonization.

The current regulation in Serbia, bearing in mind the transition period, is constantly subject to changeThe four most important groups of laws, ordinances, regulations and various decisions that regulate or relate to the field of agriculture and that are significant for the preparation and application of Good Agricultural Practices in Serbia are the laws and regulations in the field of water management and protection and environmental protection, regulations from the areas of plant health and nutrition, regulations in the area of animal health and legislation in the area of biodiversity and landscape conservation.

Each of these groups of regulations is largely harmonized with EU regulations. At the same time, it is imperative to achieve the appropriate quality of all water within the national framework, which is increasingly becoming a condition of survival, but also a very big obligation of the state immediately after the introduction of the European Water Framework Directive and the Nitrate Directive into the national legislation.

An important document, a kind of manual that should consolidate all obligations of agricultural producers related to environmental protection and provide explanations and rules for what, why and how they should work on their farms, is the Code of Good Agricultural Practices of Serbia (Rules to the Code of Good Agricultural Practice, 2023), one of the documents that our country must have before joining the EU and which, in cases prescribed by law, will also have mandatory application on agricultural farms.

„The rules of good agricultural practice for the management of agricultural waste and organic waste in Serbia aim to promote environmentally acceptable practices among farmers in Serbia. The PDPP includes practical measures that lead to the reduction of nitrogen and phosphorus discharges into surface and groundwater. The proposed measures will also reduce ammonia emissions, reduce the risk when using pesticides and reduce soil degradation. The proposed measures are based on the existing regulation in Serbia and the one in the adoption and preparation procedure. The proposed PDPP for Serbia is also based on the existing PDPP from other European Union countries" (Conclusion on the adoption of the IPARD Program for the Republic of Serbia for the period 2014-2020. years, 2017).

The documents adopted by Serbia are the Water Management Strategy on the territory of the Republic of Serbia until 2034 (Water management strategy on the territory of the Republic of Serbia until 2034, 2017) and the Law on Amendments to the Law on Water (Law on Amendments to the Law on Water, 2016). Legislation related to water in the Republic of Serbia is not fully harmonized with EU legislation even after the adoption of amendments to the Law on Water. The water management plan for the Danube basin on the territory of the Republic of Serbia has not been adopted. The drafting of the new Law on Water is underway, which will fully implement the EU legislation related to water, and then, in accordance with that new Law, water management plans will be drawn up, time-aligned with the third cycle of drawing up management plans for river basins in the EU (2022 -2027). The Ministry of Agriculture, Forestry and Water Management has formed a working group for the development of elements for the preparation of water management plans, in which the civil sector is also involved. The working group has held one working meeting so far (Coalition 27, 2018).

Conclusion

Soil is a natural resource that is being consumed at a high rate, primarily due to the modern way of agricultural production. The public does not know enough about this, nor is there adequate care for soil protection. Although soil is a renewable natural resource, soil renewal is occurring at a rate many times slower than the current degradation and loss of soil fertility.

For the purposes of implementing the Sofia Declaration, an Action Plan for the implementation of the "Green Agenda for the Western Balkans" was drawn up, which defines a road map within area III Reduction of air, water and soil pollution in the Western Balkans - the signatory countries of the Sofia Declaration will jointly direct their activities with the EU in order to implement the relevant legal acquis of the EU in the field of water (the EU Water Framework Directive, the Urban Wastewater Treatment Directive and the Nitrate Directive), the modernization of the infrastructure for water monitoring, as well as the construction of the necessary infrastructure for the treatment of waste water. The action plan establishes a framework for coordination and support for proper implementation and monitoring of the implementation of the "Green Agenda for the Western Balkans" (Serbia and Agenda 2030, 2022).

The National Environmental Approximation Strategy for the Republic of Serbia unifies, rationalizes and expands the existing framework for planning the transposition of EU regulations, strengthens implementation, control and supervision and provides the infrastructure needed by the Republic of Serbia, its municipalities, businessmen and citizens, in order to enable their alignment with EU environmental regulations. Accordingly, in the area of water, the National Approximation Strategy defines the scope of work and costs necessary for compliance with the Water Framework Directive, the Wastewater Treatment Directive, as well as the Flood Risk Directive and the Hazardous Substances Release Directive (Directive 2006/118/EC), The Freshwater Fish Directive, the Bathing Water Directive (Directive 2006/7/EC), The Wastewater Treatment Directive and the Nitrate Directive, for which there is currently no equivalent in the legislation of the Republic of Serbia. Realization of the necessary activities that will ensure harmonization of national legislation with EU directives and provide conditions for their effective implementation will enable the achievement of sub-goal 6.5. Implement integrated management of water resources at all levels (Serbia and Agenda 2030, 2022).

By introducing philonic responsibility, Kant's categorical imperative is clearly extended to the biotic community as a whole, which also includes numerous life forms of the biotic community that are found in the fertile soil and provide the opportunity for life to grow on it and in it. From this we can conclude that in our responsible attitude towards agricultural land, we should take into account the entire biotic community and not only the "possible" well-being of humans (Čović, 2004).

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BIOCHEMICAL RESEARCH OF THE SPECIES *ORCHIS MORIO* L. FROM ZLATAR

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Abstract: Salep tuber is sourced from young tubers of plants belonging to genera such as *Orchis (Orchis morio* L, *Orchis militaris* L, *Orchis mascula* L, *Orchis latifolia* L), *Ophrys* (*Ophrys fuciflora* (Cr) Haller), *Platanthera* (*Platanthera bifolia* (L) Rich,), *Anacamptis* (*Anacamptis pyramidalis* (L) Rich), and *Gymnadenia* (*Gymnadenia conopsea* (L) RBr) from the Orchidaceae family. Ph.Yug.II categorizes *Orchis morio* L as salep, aligning with CITES convention and IUCN categories for vulnerable species. As a mucilaginous drug, salep is used as an enema for intestinal catarrh and added to drugs with local irritating effects due to its soothing impact on mucous membranes. Qualitative gas chromatographic-mass spectrometric (GH/MS) analysis of Orchis morio tubers from Zlatar mountain's dry meadows reveals compounds like cinnamaldehyde, α-terpinyl acetate, coumarin, α-muurolol, pogostol, caryophyllene, n-hexadecanoic acid, linoleic acid, pentacosane, heptacosane, nonacosane, campesterol, cyclolartenol, sitosterol, and predominantly β-sitosterol. The elevated β-sitosterol content supports the use of salep in treating benign prostate diseases.

Keywords: *Orchis morio* L., Salep tuber, β-sitosterol

Introduction

The source of the drug Salep tuber are young tubers of plants from the Orchidaceae family. The plant species we used are *Orchis morio* L., *Orchis militaris* L., *Orchis mascula* L., *Orchis latifolia* L., *Ophrys fuciflora* (Cr.) Haller *Platanthera bifolia* (L.) Rich, Anacamptis pyramidalis (L.) Rich, and *Gymnadenia conopea* (L.) R B. The following species are listed on the CITES list of protected

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plants in Serbia and Montenegro: *Anacamptis pyramidalis* (L) Rich, *Gymnadenia conopsea* (L) RBr, *Orchis militaris* L, *Orchis morio* L According to EU laws on trade in wild plants, these species are protected and listed for SCG (EU Regulation on trade in wild animals Protection of wild fauna and flora species by regulating trade in them). The Ph.Yug. II mentions the *Orchis morio* L. type as salep, which is in accordance with the CITES convention and IUCN vulnerable species categories. It is widespread in the following areas: Europe, northern part of England, southern part of Norway and Estonia, southeastern and central Russia, which are areas rich in this plant species. *Orchis morio* L. belongs to the Mediterranean-Submediterranean-Pannonian Atlantic-Western Sarmatian floristic element (Obratov-Petkovic, et al., 2007; Bogosavljević et al., 2014). It is widespread in Europe, areas rich in this plant species are the northern part of England, southern part of Norway and Estonia, southeastern and central Russia. In southern Europe, this mesothermic species is widespread in mountainous areas. In Serbia it is represented on mountains Goč, Kopaonik, Tara, Golija, Javor, Mučanj, Ovčar-Kablar gorge, gorge of Svrljiški Timok, Carska bara - old Begej (altitude 75-85m).

Orchis morio L. is a perennial herbaceous plant that inhabits extremely dry habitats, but can also be found in mesophilic phytocenoses, which is why it is classified as a subxerophyte ecological group. The soil on which it grows has neutral to weakly acidic pH values, and a small amount of nitrogen in the soil is sufficient for its growth and development. The results of the research pointed to the harmful consequences of even small amounts of fertilizers (containing N, P, K and Mg) and it is not recommended to use either organic or inorganic fertilizer in meadows where it is necessary to preserve *Orchis morio* L. (Silvertown et al., 1994). It has been determined by analyzing the buds of European orchids that they contain cyanidinglucoside (orchicyanin). As the concentration of this anthocyanin is specific to each species and genus, identification of orchids can be performed by determining the concentration of anthocyanin during flower development (Uphoff, 1981).The tuber *Orchis morio* L. are round, rarely oval. The tuber contains 50% mucus, 25% starch, 5% protein, 2% cellulose, 1% sugar, a little bitter substances, fats and tartaric acid. Research indicates that salep samples obtained from tubers of *Orchis anatolica*, *Orchis italica*, *Orchis morio*, *Orchis tridentata* and *Serapias vomeracea ssp orientalis* contain higher amounts of mucus and have higher viscosity than those obtained from tubers of *Dactylorhiza osmanica* var *osmanica*, *Ophrys mammosa*, *Orchis coriophora*, *Orchis palustris*, *Orchis simian* (Tekinşen & Güner, 2010).

Salep as a typical mucous drug (Mucilago) is used as an enema in the intestinal catar and as an adjunct to drugs with a locally irritating effect because it soothes mucous irritation (Tekinşen & Güner, 2010).

Materials and methods

Plant material (tubers) was collected from dry meadows on The Zlatar Mountain. After identification, the sample was left to dry naturally, and then packed in a paper bag. For the purposes of analysis, the plant is protected.

Gas chromatography-mass spectrometry analysis (GC/MS) was performed using an Agilent 6890 gas chromatograph connected to an Agilent 5973 Network mass selective (MSD) and flame ionization detector (FID) in positive electron impact (EI) ionization mode. The separation was carried out on the Agilent 19091S-433 HP-5MS capillary column, measuring 30 m x 0.25 mm i.d. and film thickness 0.25 μm. GC/MS analysis was performed under the following conditions: temperature range of 60-285°C (4.3°C/min); carrying helium gas with a flow rate of 1 ml/min, measured at 210 °C; injector temperature 250 ° C, and injection in splitless mode. Conditions of MS analysis: source temperature 200 °C; interface temperature of 250°C; ionization energy of 70 eV; mass shooting range 40–350 amu (atomic mass units).The quantification of individual components was performed using a flame ionization detector. By comparing the areas of the graph obtained by analyzing the sample and the internal standard, the relative proportion of each component was calculated. Component identification was performed by comparing the results of GH/MS analysis with spectra (NIST and Wiley and Adams library). The ^{1}H NMR spectrum was recorded on a Varian Gemini 2000 r (200 MHz) camera. DMSO-d6 and TMS were used as an internal standard.

Results and discussion

The results of the qualitative gas chromatography-mass spectrometry analysis are shown on the chromatogram (Figure 1). Signals from the detector on the graph are shown as a series of peaks, each representing an individual component from the mixture of the tested sample, while the area under the peak represents the amount of the present component in ppm (parts per million, mg/l).

Signals on a chemical shift from 3.00 to 5.20 δ indicate the presence of sugar, and signals between 6.8 and 8.1 δ the presence of compounds with aromatic rings. The results of the qualitative analysis are shown in Figure 2.

Figure 2. Amounts of components present in the *Orchis morio* L. tuber sample in ppm

Component mixtures after separation show different retention times -times for each component in the column. Compounds shown in Table 1. were identified by their detection, retention time is given for each component, as well as the relative proportion in the sample of the plant drug salep.

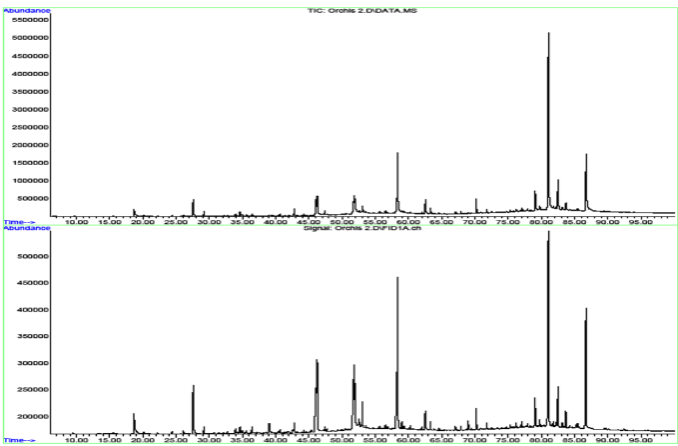

Figure 1. Chemical components in tubers of the species *Orchis morio* L.

Identification compounds of the peaks in the chromatogram shown in Figure 1 and 2 are represented in Table 1. The results show that of all compounds, the highest relative proportion is β-sitosterol and linolenic acid. Sterols are essential components of the cell membrane and animal and plant cells. Pharmacological screening of β-sitosterol has been established to possess numerous activities such as antimicrobial, anti-inflammatory, anticancer, antifertility, antioxidant, antidiabetic (Chanioti at al., 2021; Le Goff et al., 2019; Ambavade at al., 2014). Several studies and clinical trials suggest the use of β-sitosterol against prostate, colon and breast cancer, experimentally these studies confirm the important protective role of β-sitosterol in cancer prevention (Chanioti at al., 2021; Le Goff et al., 2019). An in vitro study shows that β-sitosterol effectively inhibits the growth of HT-29 cancer cells tumors (a line of human colon cancer cells) (Jones & Abumweis 2009). Due to its positive properties, β-sitosterol is used in herbal therapy and in the treatment of benign prostatic hypertrophy). Alone or in combination with similar phytosterols β-sitosterol also reduces the level of cholesterol in the human blood, hence it can be used in the treatment of hypercholesterolemia, inhibiting cholesterol absorption in the intestinal tract (Matsuoka et al., 2008).

Table 1. Identification compounds in a salep obtained from *Orchis morio* L. Tubers

Jedinjenja Compound	Retenciono vreme Retention time	Relativni udeo Relative abundance
Cinamaldehid	18.68	3.077
α-terpinil acetat	22.16	0.187
Kumarin	26.03	0.731
α-murolol	34.59	0.890
Pogostol	34.93	0.449
Cariofilen	35.60	0.298
n-heksadekanska kiselina	46.23	10.458
Linolenska kiselina	51.79	15.300
ND	51.98	5.578
Pentakosan	61.95	0.221
ND	62.5	2.256
Heptakosan	67.07	0.290
Nonakosan	71.78	trag
Campesterol	79.12	3.924
β-sitosterol	80.94	28.266
Cikloartenol	82.46	4.172
Sitostenon	83.67	1.773

Another component that is found in a large concentration is linoleic acid. Linoleic acid enters the membrane of plants, for animals and humans is an essential fatty acid, necessary for a number of metabolic processes and the normal functioning of the body and the preservation of health as a whole. Linoleic acid deficiency causes hair loss, poor wound healing. Its consistent intake is associated with a reduced risk of atherosclerosis, hypercholesterolemia (Das, 2021; Ramsden et al., 2021). and other chronic health conditions.

Conclusion

Based on the performed qualitative gasnochromatographic-mass spectrometric (GH/MS) analysis, it can be concluded that tubers of the species *Orhis morio* L. collected from the dry meadows of The Zlatar Mountain contain:

cinnamaldehyde, α-terpinyl acetate, coumarin, α-murolol, pogostol, caryophyllene, n-hexadekanic acid, acid, pentacosan, heptacosan, noncosan, campesterol, cycloartenol, sitostenone, and in the largest quantities β-sitosterol and linolenic acid. Large amounts of β-sitosterol sytosterol indicate the justification of the use of salep in pharmacotherapy in the treatment of benign prostate diseases, but also certain types of cancers such as prostate, colon and breast cancer.

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BIOLOGIAL ACTIVITY OF PLANT METABOLITES

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Abstract: As part of theoretical and applied botanical research, medicinal plant raw materials and their ingredients are studied. Certain plant species contain active substances, compounds with proven pharmacological, that is, biological activity. The mentioned compounds mainly belong to the secondary metabolites of plants.

Keywords: botany, plants, plant metabolism, biological activity.

Introduction

Given that plants have influenced the historical development of civilization, botanical research has enormous theoretical and practical importance. As part of theoretical and applied botanical research, medicinal plant raw materials are studied. The chemical structure of the most important groups of pharmacologically active compounds of plant origin, the production and cultivation of medicinal plants, as well as their pharmacological, i.e. biological action and application, are studied. Also, knowledge and skills of identification and control of natural raw materials, knowledge of phytopreparations, and possibilities of rational phytotherapy are acquired. Herbal drugs and herbal preparations contain active compounds with proven pharmacological activity. The therapeutic effect of plant species is based on active ingredients, which most often belong to the group of secondary metabolites (Jančić, 2004; Kovačević, 2004; Šarčević-Todosijević et al., 2018; Šarčević-Todosijević et al., 2023).

In this paper, the biological activity of plant metabolites is discussed.

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Materials and methods

The method of data analysis, which was collected from certain studies, was applied. Relevant scientific data, published in scientific books, papers in international and national scientific journals and scientific conferences, were used. The analyzed scientific papers belong to the categories of original and review papers.

Results and discussion

Through scientific research work in the field of biology, pharmacy and agronomy, pharmaceutical, agronomic, chemical and biological aspects of medicinal and aromatic plants are studied. Research on the quality and resources of wild plants, chemical analysis of plants and their extracts using modern analytical methods, as well as testing of biological activity in order to confirm the effectiveness of plants used in traditional phytotherapy is carried out. These researches are also carried out in order to find potential new medicinal properties of plants (Kovačević, 2004; Šarčević-Todosijević et al., 2018; Šarčević-Todosijević et al., 2023).

In living organisms, a large number of biochemical reactions, catalyzed by specific enzymes, are continuously carried out. The primary metabolism of a plant cell includes the process of photosynthesis and the subsequent transformation of glucose into polysaccharides, lipids and proteins. Secondary metabolism is a direct continuation of primary metabolic processes, and compounds of a very diverse chemical nature are formed: alkaloids, heterosides, saponosides, polyphenols, etc. The use of medicinal plants is regulated by the pharmacopoeia, which represents a collection of prescribed norms and standards for substances and the manufacture of medicines, which determine their identification, characteristics, quality, method of preparation and analysis. Herbal drugs are medicinal raw materials of plant origin, which contain pharmacologically active compounds and are used for the extraction or production of medicinal preparations (Jančić, 2004; Kovačević, 2004; Šarčević-Todosijević et al., 2019a).

Herbal drugs and herbal preparations contain compounds that have been proven to exhibit biological activity. This activity is not expressed for primary plant metabolites, carbohydrates, proteins and lipids, while secondary plant metabolites exhibit strong and specific biological activity on the human organism, as well as other organisms. In contrast to the large application in the pharmaceutical and food industry, carbohydrates and herbal drugs containing them have a very limited therapeutic application. Because of its nutritional value, glucose is given to patients as an infusion. Plant proteins also rarely have pharmacological activity and a certain therapeutic value, except that, like glucose, amino acids mixtures are prepared and given by infusion to certain groups of patients. In addition, enzymes isolated from plant tissues are used in therapy (Kovačević, 2004, Šarčević-Todosijević et al., 2019b, Popović et al., 2021; Petrović et al., 2022).

From the aspect of lipid application in pharmacy, it is necessary to mention solid lipid nanoparticles (LNPs), as a new pharmaceutical drug delivery system. Solid lipid nanoparticles (LNPs) have been used more intensively since the end of 2020. Some COVID-19 vaccines have fragile mRNA chains coated with lipid nanoparticles as a delivery vehicle. Oral administration of LNPs is possible in the form of an aqueous dispersion or after conversion into traditional pharmaceutical forms, e.g. tablets, capsules or powders. Solid lipid nanoparticles can be transformed into a powder and added to a mixture of tableting powders. Some therapeutic problems, such as instability, low membrane permeability and drug bioavailability, can be solved by designing appropriate delivery systems based on the combination of two basic classes of ingredients: polymers and lipids (Milić et al., 2005).

The use of most biologically active substances is more recent, when their powerful medicinal or protective effects (antioxidant, antimutagenic, anticarcinogenic) were confirmed. One of the most important such compounds is a diterpene alkaloid taxol, which is produced by *Taxus brevifolia* and *Taxus baccata* plant species. It is used in the therapy of cancer metastases. Alkaloids are versatile heterocyclic nitrogen compounds, products of the secondary metabolism of plants, which exhibit a strong and specific pharmacological activity on the human and animal organisms (Kovačević, 2004; Šarčević-Todosijević et al., 2019a). Despite long-term use, there is still an interest in testing new unexplored herbal drugs, potential raw materials for the pharmaceutical industry, for more detailed testing and definition of bioactive content, as well as for the partial synthesis of new alkaloid molecules based on natural alkaloids (Tanović, 2005). Expectorants are drugs that increase pulmonary secretion. In addition to synthetic expectorants, herbal drugs with an expectorant effect are also used, especially in self-medication (Halliwell, 1997). Ipecacuanhae root, *Ipecacuanhae radix*, is the only alkaloid drug that is used as an expectorant. After oral administration, emetine irritates the mucous membrane of the stomach and through a reflex pathway, via afferent parasympathetic fibers, stimulates the glandular cells of the bronchial mucosa and increases the secretion (Husain et al., 1987). In addition to a number of ingredients, compounds from the group of alkaloids were detected in *Achillea millefolium*, namely: achillein, achilicine, betaine. Experiments have shown that achillein detected in *Achillea millefolium* has hemostatic and antimicrobial properties. In addition to alkaloids, as biologically active ingredients, the essential oils of this plant also exhibit antioxidant and antimicrobial properties (Aryal et al., 2022). As mentioned, most secondary metabolites do not directly participate in the growth and development of plants and are not necessary for their life, but they play a major role in the interaction of plants with the environment. This group of plant metabolites also includes polyphenols, a class of natural, synthetic, and semisynthetic organic compounds. They include phenolic acids, flavonoids, coumarins, stilbenes and lignans, but also other polymerized forms, such as tannins and lignins. As natural products of the secondary metabolism of plants, they are found in fruits, vegetables, nuts and spices (Kovačević, 2004; Mattila et al., 2006; Ćetković, 2008; Spencer et al., 2008). The intake of vegetables, fruits and cereals is associated with a reduced risk of developing chronic diseases, primarily thanks to plant metabolites, which reduce oxidative stress in cells, which causes damage to biological macromolecules (DNA, lipids and proteins), which results in the appearance of degenerative diseases (Temple, 2000). Based on *in vitro* studies, it was concluded that the effect of red wine polyphenols on vascular tone is due to short-term and long-term mechanisms. Vasorelaxation is a short-term response to wine polyphenols, which act by increasing the influx of extracellular Ca and mobilizing intracellular Ca in endothelial cells. Polyphenolic compounds can also exert long-term effects, because they increase the expression of endothelial nitric oxide synthase, decrease the expression of adhesion molecules and growth factors involved in the migration and proliferation of vascular smooth muscle cells (Dell'Agli, 2004). The use of herbs is one of the most popular and common ways to reduce menopause symptoms (Geller and Studee, 2006). Numerous studies have shown that regular consumption of phytoestrogens in the diet of Asian women leads to a reduction of menopausal symptoms (Adlercreutz et al., 1992). Mohammad-Alizadeh-Charandabi et al. (2013) conducted a randomized, double-blind, placebo-controlled trial. 84 women were randomly assigned to treatment and control groups. The results of this study indicated that taking black cohosh (*Actaea racemosa*) for 8 weeks, in the form of one tablet after dinner (tablet contains 6.5 mg of extract as dry extract), reduced symptoms in early postmenopausal women compared to placebo. The effectiveness of the treatment increased with longer use (Mohammad-Alizadeh-Charandabi et al., 2013).

Conclusion

As part of theoretical and applied botanical research, medicinal plant raw materials, their ingredients, chemical structure, production and cultivation of medicinal plants, as well as their pharmacological, i.e. biological activity and application, are studied. The most pronounced biological activity has been scientifically proven for the products of the secondary metabolism of plants.

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BIODEGRADABILITY ASSESSMENT OF CORN STOVER REINFORCED COMPOSITE MATERIALS WITH DIFFERENT MATRIX

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Abstract: In this paper, the biodegradability of three SferiCorn™ biocomposites was investigated. Corn stover prepared through eco-friendly washing and grinding to short fibers was used as reinforcement. Three different biopolymers, corn starch, alginate and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), were used as matrices. The biodegradability of prepared composites was investigated in a simulated soil burial test and the samples were characterized in terms of weight loss and morphological changes. It was shown that biodegradability can be tuned by biopolymers used as matrices. Changes in the surface morphology after biodegradation of tested samples were confirmed using scanning electron microscopy (SEM).

Keywords: corn stover composites, bio-polymer matrix, biodegradability, composting

Introduction

Agricultural waste, often referred to as agri-waste, is the byproduct generated from various agricultural activities. It includes residues, byproducts, and non-food parts of crops that are left behind after harvest or processing (Duque-Acevedo et al., 2020). Agro-waste can be substantial, and its management is crucial for environmental sustainability (Phiri R. Et al., 2024). Corn stover composite refers to a material that is produced by combining corn stover, which is the residual biomass left in the field after corn harvest, with a matrix material to create a composite material with enhanced properties (Mohammed A. A., 2022). Utilizing corn stover in composite materials is a

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sustainable approach as it repurposes agricultural waste, reduces environmental impact, and provides an alternative to synthetic composites materials (Suess T., 2013). Some common types of matrix materials used in corn stover composites are thermoplastic polyolefins from the recycling industry, such as polyethylene (Chun K. S. Et al., 2017), or polypropylene (Delgado-Aguilar M. et al., 2018) on one hand, or, on the other hand, polyvinyl alcohol very well known for its biodegradability (Yong K. J., 2022). A biodegradable and bio-based polymers such as polylactide acid (Qi Z., 2022), polycaprolactone (Wu C. S. and Liao, H. T. 2012), polyurethane (Kocak E. D. et al., 2023), natural resins such as soy-based resins (Pfister D. P. and Larock R. C. 2010), starch-based (Ibrahim M. I. J. et al. 2019) or lignin-based matrix (Yuan Y. et al., 2019) that enhances the eco-friendliness and sustainability of corn stover composites, can be used. Combining different polymers to create a hybrid matrix that optimizes properties such as strength, flexibility, and biodegradability, polymer blends are also a possible choice (Li R. et al. 2023). Among the very interesting polymers for the role of the matrix in corn stover composites are a family of biodegradable polyesters synthesized by a variety of microorganisms as intracellular carbon and energy storage compounds named polyhydroxyalkanoates (PHAs). PHAs are considered promising bioplastics due to their biodegradability, thermoplastic properties, and potential to replace conventional petroleum-based plastics in many applications, among which are thermoplastic matrices for composite materials (Sharma V. et al, 2021). PHAs are biodegradable and can be synthesized with various monomer compositions, leading to a range of materials with different physical and mechanical properties (McAdam B. et al., 2020). Due to their thermoplastic properties, can be processed using conventional plastic processing techniques such as extrusion, injection molding, and film blowing (Raturi G. et al., 2021). In this paper, the degradation possibilities of three SferiCorn™ biocomposite materials with corn stover ligno-cellulosic reinforcement and different polymer matrices are considered. The potential impact of the study extends to the realms of waste management, environmental sustainability, and the development of green alternatives to traditional synthetic composites.

Materials and methods

SferiCorn™ composites. Three types of SferiCorn™ composite materials with the same reinforcement and different matrices were made. The composition and properties of individual composites are given in Table 1. The structure of the composite is quasi-isotropic in all three tested kinds of samples.

Corn stover is provided from Agricultural Farm Marko Adžić from Vojvodina, Republic of Serbia, Corn Starch from Klas plus d.o.o. Republic of Srbija, and Sodium Alginate from Cenik Chemicals UK. Aonilex® X131A PHBH was supplied by Kaneka, Japan.

Table 1. Composition and the properties of prepared samples

Samples name	SferiCorn R	SferiCorn S	SferiCorn BioTP
Reinforcement	Ground corn stover		
Matrix	Starch 12.5%	Alginate 2%	Aonilex® X131A
Matrix/ reinforcement ratio	1:1	1:1	1:1
Areal density, kg/m2	1.4	0.9	4.1
Surface	Rough	Rough	Smooth

The production processes of SferiCorn™ composites are completely eco-friendly, including the corn stover washing process. All three composite materials were produced using different technological procedures, according to the requirements imposed by composite matrices.

Simulated soil burial biodegradation tests. Simulated soil burial biodegradation test was done following the procedure: compost and garden soil were mixed (1:1) and mixed with water (3:1) in a Petri dish (20 cm diameter, 3 cm hight) and the tested samples (rectangular shape, 1 x 1 cm^2) were buried at depth of 1 cm. Samples were incubated for 10 days and 17 days, and after predetermined time intervals samples were washed with water, and dried in a vacuum oven to achieve constant mass and the weight loss was calculated according to the equation (1):

$$\textit{Weight loss (\%)} = (m_1 - m_0)/m_0 \times 100\ \% \quad (1),$$

Where m_1 is remained mass after degradation and m_0 is the starting mass of the samples.

Scanning Electron Microscopy (SEM) analysis. The surface morphology of the control samples, but also changes in morphology after the biodegradation test in soil were investigated using scanning electron microscopy (SEM, JEOL JSM-6390LV JEOL USA Inc., Peabody, MA, USA) applying an accelerating voltage of 15 kV. Prior to the recording at various magnifications, all samples were fixed on supports and sputter-coated with a thin layer of gold.

Results and discussion

Weight loss after simulated soil burial biodegradation test. The biodegradation ability of the SferiCorn™ samples were quantified by measuring weight loss after predefined time intervals (Figure 1). After 10 days of degradation, a significant weight loss, of almost 80 %, was detected for the SferoCorn R sample, while the SferiCorn S composite with alginate appeared to be more stable under the testing conditions and lost more than 40 % of its mass. With the extension of the degradation time, samples were mostly disintegrated with a weight loss close to 100 % indicating that SferiCorn™ samples are highly compostable, biodegradable material. However, the composite of SferiCorn samples with PHBV showed better stability under the tested conditions with the weight loss of only a few percentages even after 17 days of composting, due to the presence of biopolymer PHBV.

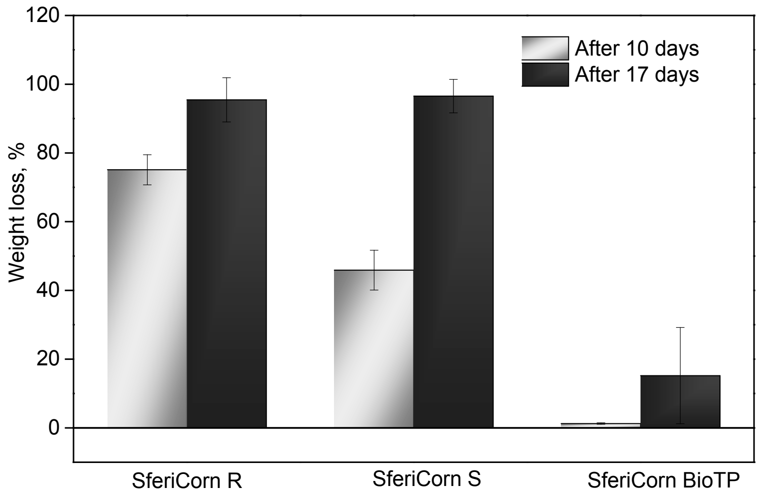

Figure 1. Weight loss of SferiCorn samples after 10 days and 17 days under composting.

SEM analysis. The surface morphology of the SferiCorn composite material before and after composting was evaluated by SEM analysis (Figure 2). Morphology of all SferiCorn control samples appeared porous with the most prominent porosity in the case of SferiCorn R, while its composites with alginate and PHBV possessed less porous and more compact morphology due to the presence of biopolymers. After the biodegradation of both 10 days and 17 days, beside remained pieces of compost were detected, the samples were remarkably disintegrated and the sponge-like morphology was less visible as a consequence of remarkable weight loss.

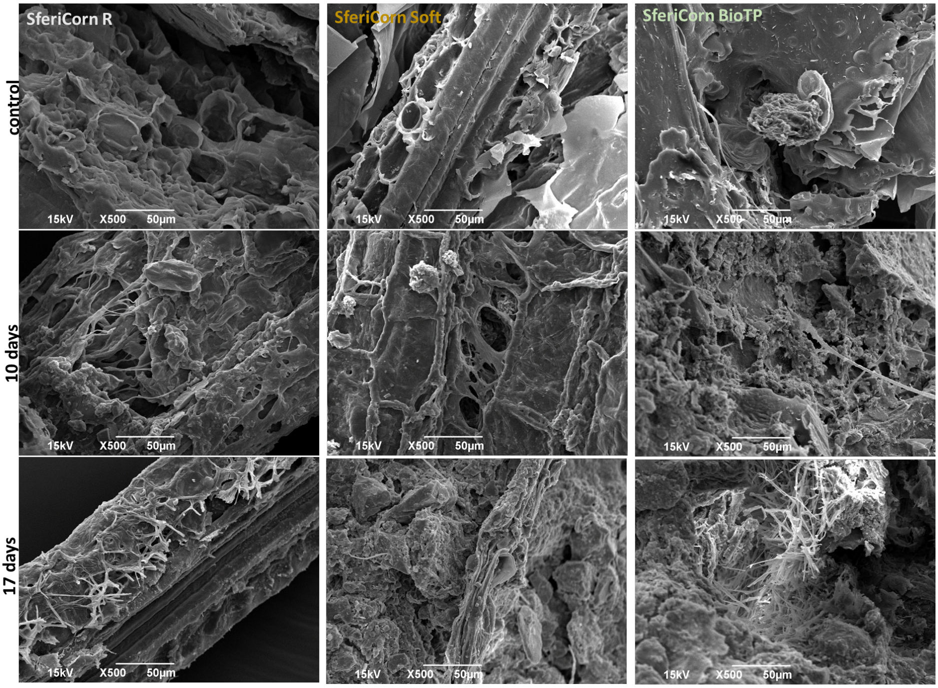

Figure 2. SEM analysis micrographs of the samples before and after biodegradation in soil after 10 days and 17 days (magnification of 500 times).

Conclusion

By analyzing the obtained results, it can be concluded that SferiCorn™ samples are highly compostable, biodegradable materials, but with different susceptibility to environmental degradation, tuned by the selected biopolymer. Therefore, the highest biodegradability was proved for the composite SferiCorn R, followed by SferiCorn S, while the SferiCorn BioTP showed the highest stability. SEM analysis of surface morphology of the SferiCorn samples before and after composting indicated a porous morphology that is most expressed in the case of SferiCorn R sample, while SferiCorn S and SferiCorn BioTP have a less porous and more compact morphology provided by the biopolymers used for composite production. After biodegradation, the samples apart of PHBV reinforced SferiCorn™ composite material, were significantly disintegrated and the sponge-like morphology was less visible as a consequence of notable weight loss. The versatility of SferiCorn™ composites extends to varied applications, contingent upon the chosen matrix. Specific properties imparted by different matrices result in tailored lifetimes, rendering each composite uniquely suited for specific applications.

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SESELI RIGIDUM WALDST. & KIT: SECONDARY METABOLITES AND ANTIOXIDANT ACTIVITY OF METHANOL, ETHYL ACETATE AND ACETONE EXTRACTS

Gorica Djelic[1], Vesna Veličković[2], Milica Pavlović[1]

Abstract: Representatives of the Seseli genus are recognized for their biological activities. The objective of this study was to analyze the phenolic and flavonoid content, along with the antioxidant effects, of methanol, ethyl acetate, and acetone extracts obtained from the roots, leaves, stems, and fruits of *S. rigidum.* Through spectrophotometric measurements of phenols and flavonoids, the results revealed that the polar (methanol) solvent extract from the leaves exhibited the highest concentrations of phenols (98.66 ± 2.64 mg GA/g) and flavonoids (20.74 ± 0.55 mg RU/g), surpassing those obtained from the stem. Specifically, the methanol extract from the leaves demonstrated the highest antioxidant activity at 25.87 ± 0.05 μg/mL, while the fruit extract exhibited the lowest antioxidant activity at 638.5 ± 0.01 μg/mL. Notably, the total phenol content displayed a strong positive correlation with the antioxidant activity of the extracts, unlike the total flavonoid content.

Keywords: *S. rigidum*, phenols, flavonoids, DPPH

Introduction

Seseli rigidum Waldst. & Kit. (Apiaceae) is a subendemic perennial plant, commonly known as "devesilje" or "devesilj" (Ilić, 2016). Previous research has identified essential oils, coumarins, flavonoids, lignans, sesquiterpene lactones, and polyacetylenes in species of the Seseli genus (Abbaskhan et al., 2012). Traditionally, Seseli genus species are used, with roots employed to alleviate pain in bones, muscles, and joints, leaves used in culinary practices, and fruits utilized as a remedy for gastrointestinal issues (Gonçalves, 2012). The essential oil extracted from Seseli genus species has demonstrated antioxidant,

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antibacterial, antifungal, anti-inflammatory, and moderate cytotoxic activities (Marčetić, 2012).

Various studies have explored the antifungal, antigenotoxic, genotoxic, and antimicrobial properties of the *S. rigidum* plant in previous research (Živković, 2016; Marčetić, 2019). The current study aims to investigate the phenolic and flavonoid content, as well as the antioxidant effects, of methanol, ethyl acetate, and acetone extracts obtained from the roots, leaves, stems, and fruits of *S. rigidum.*

Materials and methods

Plant material and extracts preparation

Plant material, *S. rigidum* was collected in July 2022 from the locality Ovcar-Kablar Gorge (43°55′07″ N; 20°13′17″ E) in the flowering phase, (fruit, stem, leaves, and root). Collected materials was cleaned, and dried in a dark room at room temperature. The dried and finely ground fruit, stem, leaves, and root (50 gr) were extracted with methanol, ethyl acetate, and acetone (250 mL). After the 24 hours plant material was filtrated, and then 250 mL of adequate solvents were added. After next 24 hours, the procedure was repeated. After extraction, the material was evaporated to dryness using a rotary evaporator at 40°C. The obtained extracts (twelve) were stored in sterile tubes at 4°C till the chemical analysis.

Determination of total phenols content of the extracts

The total phenol content of the sample's methanol solutions was measured by the Folin-Ciocalteu assay as described by Peter et al. (2011). The absorbance was measured using a spectrophotometer at λ max = 725 nm. Gallic acid (GA) was used to construct the standard curve, which showed the linear regression of $r^2 > 0.99$, and the values obtained for the concentration of total phenols are expressed as GA/g of extract.

Determination of total flavonoid content of the extracts

The concentration of flavonoids in the sample's methanol solutions was measured by the method of Quettier-Deleu et al. (2000). The absorbance was measured using a spectrophotometer at λ max = 430 nm. Rutin (RU) was used to construct the standard curve, which showed the linear regression of $r^2 > 0.99$, and the values obtained for the concentration of total flavonoids are expressed as RU/g of extract.

Determination of the antioxidant activity of the extracts

The antioxidant activity of the plant extracts against DPPH was determined using the method proposed by Takao et al. (1994). Ascorbic acid was used as the standard. The absorbance was measured at λ max =517 nm. The percent DPPH inhibition was calculated by the equation (Eq. 1). Antioxidant activity was expressed as the 50% inhibitory concentration (IC_{50} values in μg/mL).

$$\%inhibition=((\ A\ of\ control - A\ of\ sample)/(A\ of\ control))x100 \quad (1)$$

Statistical analysis

The content of phenols, flavonoids and antioxidant activity were carried out in triplicate and expressed as the average value, ± standard deviation. Statistical analyses of data was made by using IBM SPSS Statistics 21.0 (2012). The collected data were analyzed by the one-way ANOVA and Tukey post-hoc test. Statistically significant difference was defined as $p < 0.05$.

Results and discussion

Total contents phenols *of the extracts*

Results of the total amount of phenols in fruits, stem, leaf and root of species *S. rigidum*, are shown in (Table 1.).

Table 1. The total amount of phenols determined in different *S.rigidum* extracts presented as equivalents of gallic acid (mg of GA/g extract)

	Fruits	**Stem**	**Leaf**	**Root**
Methanol	61.94 ± 0.25^b	98.66 ± 2.64^a	95.06 ± 0.54^a	75.38 ± 2.83^b
Ethyl-acetate	71.06 ± 1.42^a	79.94 ± 3.09^b	59.67 ± 5.36^c	64.83 ± 3.61^c
Acetone	61.89 ± 0.25^b	80.72 ± 1.13^c	81.845 ± 1.36^b	85.11 ± 1.71^a

Values are mean ± standard deviation of triplicate analyses. Values within the row followed by the same letter (a, b, c), are not significantly different according to Tukey's test (p<0.05).

The highest concentration of phenols was extracted with polar solvents - methanol (98.66 ± 2.64 mg GA/g) from stem, and (95.06 ± 0.54 mg GA/g) from leaves, and the lowest concentration of phenols was measured in extracts obtained with non-polar solvent (ethyl-acetate) (59.67 ± 5.36 mg GA/g) from leaves. Differences in phenol content among extracts made by different solvent were significant ($p < 0.05$). Compared to the results published by Ilić, (2017), in the polar solvent acetone constated the highest amount of phenol (151.03 ± 4.47) was found in the root, and the lowest concentration coincides with the results of our study, (ethyl-acetate) (3.96 ± 0.12 mg GA/g) from leaves.

Total contents flavonoids of the extracts

Results of the total amount of flavonoids in fruits, stem, leaf and root of species *S. rigidum*, are shown in (Table 2.).

Table 2. The total amount of flavonoids determined in different *S.rigidum* extracts presented as equivalents of rutin (mg of RU/g extract)

	Fruits	**Stem**	**Leaf**	**Root**
Methanol	11.38 ± 0.18^{a}	20.74 ± 0.55^{a}	20.07 ± 0.63^{a}	14.33 ± 0.14^{a}
Ethyl-acetate	2.86 ± 0.38^{c}	14.98 ± 0.48^{c}	18.29 ± 0.38^{c}	14.83 ± 0.27^{a}
Acetone	9.45 ± 0.32^{b}	17.67 ± 0.22^{b}	19.95 ± 0.72^{b}	12.24 ± 0.39^{b}

Values are mean ± standard deviation of triplicate analyses. Values within the row followed by the same letter (a, b, c), are not significantly different according to Tukey's test ($p<0.05$).

The highest concentration of flavonoids was extracted with polar solvent - methanol (20.74 ± 0.55 mg RU/g) from stem, and (20.07 ± 0.63 mg RU/g) from leave, while the lowest concentration of flavonoids was measured in extracts obtained with non-polar solvent (ethyl-acetate) (2.86 ± 0.38 mg RU/g) from fruit. Differences in flavonoid content among extracts made by different solvent were significant ($p < 0.05$). Compared to the results published by Ilić, (2017), the highest concentration of flavonoids was extracted with polar solvent methanol (20.74 ± 0.55 mg RU/g) from fruit, and the lowest concentration coincides with the results of our study, (ethyl-acetate) (0.15 ± 0.01 mg RU/g) from leaves.

The antioxidant activity of the extracts

The antioxidant activity is expressed in terms of IC_{50} (μg/mL) values (Table 3). The lower IC_{50} value reflects greater activity. The highest antioxidant activity was measured in methanol (25.87 ± 0.05; 28.25 ± 3.56 μg/mL, respectively) from leave, while the lowest capacity to neutralized DPPH radicals was measured in the fruit methanol extract (638.5 ± 0.01 μg/mL). Unlike total flavonoid content, the total content of phenol is highly correlated with the antioxidant activity of extracts. Differences between antioxidant activity of extracts gained by different extraction solvents were significant ($p<0.05$). Compared to the results published by Maleki (2023), he methanolic extract also showed significant antioxidative activity (98.95 μg/ml).

Table 3. Antioxidant activity of investigated *S.rigidum* extracts, IC_{50} values (μg/mL)

	Fruits	**Stem**	**Leaf**	**Root**
Methanol	638.5 ± 0.01^{a}	69.05 ± 0.45^{c}	25.87 ± 0.05^{c}	173.95 ± 0.05^{a}
Ethyl-acetate	455.65 ± 0.01^{b}	130.4 ± 0.45^{a}	28.25 ± 3.56^{b}	138.05 ± 3.56^{b}
Acetone	375.31 ± 1.92^{c}	75.55 ± 1.21^{b}	59.21 ± 2.36^{a}	72.57 ± 2.36^{c}

Values are mean ± standard deviation of triplicate analyses. Values within the row followed by the same letter (a, b, c), are not significantly different according to Tukey's test ($p<0.05$)

Conclusions

Polar solvents showed to be more efficient in the extraction of active components, and the highest amount of tested compounds was extracted with methanol. The results showed that the leaves contains the most extracted phenols and have hight antioxidant potential, while the lowest amount flavonoids constated in from fruit. Unlike total flavonoid content, the total content of phenol is highly correlated with the antioxidant activity of extracts.

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POSSIBILITY OF USING EGG SHELL AS A FLOCCULANT TO HARVEST BIOMASS OF *Bacillus* sp. FOLLOWING BIOTECHNOLOGICAL TREATMENT OF FOOD SECTOR WASTEWATERS

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Abstract: The aim of this study was to asses the ability of egg shell flocculation to recover bacterial *Bacillus* sp. BioSol021 biomass from the broth after biological treatment of wastewaters from the dairy, wine and fruit juice industries. Different concentrations of flocculant were used to estimate the effect of egg shell dosage on flocculation efficiency and antimicrobial activity against *Aspergillus flavus*, i.e., inhibition zone diameter. The maximum values for flocculation efficiency and inhibition zone diameter (antimicrobial activity against *Aspergillus flavus*) for cheese whey effluent were 94.02%, 45 mm, respectively. The maximum values, in the case of winery flotation effluent were 87.77%, 33 mm, and for the fruit juice wastwater 87.25%, 58 mm, respectively. These results indicate that egg shells could be successfully applied as the flocculating agent for separation of *Bacillus* sp. BioSol021 from the cultivation broth.

Keywords: food industry wastewaters, microbial biomass, egg shell, flocculation

Introduction

Over the past years people have become increasingly concerned about the careless use of synthetic pesticides that have posed serious health risks to humans and the environment. Consequently, in recent decades, there has been a growing demand for food safety and quality standards, which reflected in the strict regulations on the amount of pesticide residues (Damalas and Koutroubas, 2018). Alternatives to the synthetic pesticides are mostly being developed in the form of natural products, i.e. biopesticides, considering the market demand for ecoproducts (Czaja et al., 2015).

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Biopesticides are naturally occuring compounds that include a number of living organisms (biocontrol organisms), including their genes or metabolites for controlling pests (Sporleder and Lacey, 2013). Microbial based biopesticides have shown great potential against plant diseases, as highly effective, selective and environmentally friendly solutions. Bacterial products with the *Bacillus* genus as active components express intensive activity against plant pathogens (Dmitrović et al., 2022).

In recent years, researchers have shifted their interests in reuse of food industry effluents as potential raw materials for microbial biopesticides production; many studies have investigated the high organic and inorganic burden of these effluents, making their treatment obligatory before disposal, as the pollution of water resources due to discharge of industrial effluents poses a serious threat to human beings and aquatic organisms (Qasim and Mane, 2013).

The dairy industry, has an important position among the food industries as it serves the need of global populace with products such as milk, cheese, butter, ghee, milk powder, etc. The excessive amount of whey released during cheese production creates sever environmental problems and even threatens human health (Sar et al., 2022; Usmani et al., 2022). Wine production effluents are being generated in large amounts from a number of activities that include cleaning of tanks, washing the floors, equipment, barrels and storage tanks (Dmitrović et al., 2022). Approximately, a winery produces between 1.3 and 1.5 kg of solid waste per liter of wine produced, 75% of which is winery wastewater (Ioannou et al., 2015). The fruit juice industry produces a large volume of wastewater (10 L of wastewater for 1 L of juice) through a number of processes including washing, soaking, blanching, packing, and extraction (Rashid et al., 2023). Fruit juice wastewater is highly contaminated with organic (sugars, organic acids) and suspendid solids, it is characterized by high chemical oxygen demand (COD), low-nutrient (nitrogen and phosphorous) concentration and low pH value (Amor et al., 2012; Rashid et al., 2023).

Microorganisms can be harvested using a variety of processes, including centrifugation, coagulation, filtration, and flocculation (Dmitrović et al., 2023). Flocculation has often been used as an efficient harvesting method that takes only a short time to harvest, does not require a large space, does not harm cells, and is a cost-effective aid to enhance the effectiveness of the downstream processes (sedimentation, centrifugal recovery, and filtration) through particle size enlargement (Dmitrović et al., 2023; Hadiyanto et al., 2021).

Current attention is shifted to the utilization of biocoagulants/bioflocculants (chitosan, starch, cellulose, tannins, egg shells) that can substitute synthetic organic polymers (Dmitrović et al., 2023). Egg shells have been suggested for use as a flocculant because they are non-toxic, non-corrosive, and easy/safe to handle. Additionaly, they are biodegradable, biocompatible, and show promising adsorption properties as well as flocculation ability. Egg shells have a high cationic charge density, and can, thus, successfully destabilize other particles by strongly adsorbing negative regions on them. The average egg shell contains about 95% of calcium carbonate and the remaining 5% include calcium phosphate, magnesium carbonate and soluble and insoluble proteins (Choi, 2015).

The present study investigated the influence of egg shells dosage on the flocculation process of *Bacillus* sp. BioSol021 broth coming from a biological treatment of wastewaters from the dairy, wine and fruit juice industries.

Materials and methods

Microorganisms. The producing microorganism used in this study was *Bacillus* sp. strain BioSol021, isolated from the rhizosphere of common beans or kidney beans (*Phaseolus vulgaris)* (Dmitrović et al., 2022). The investigated phytopathogen was *Aspergillus flavus* strain SA2B SS isolated from maize with proven aflatoxigenic potential (Vlajkov et al., 2021). Antagonist and phytopathogen were transferred to nutrient agar and SMA medium, respectively, and incubated under the following conditions: *Bacillus* sp. BioSol021 at 28°C for 48 h; *Aspergillus* spp. at 26°C for 120 h, in order to stimulate their metabolic activity and ability to reproduce.

Cultivation media. Cultivations of the producing microorganism were performed using media based on cheese whey (CW) as the dairy industry effluent, winery flotation wastewater (WFW), and wastewater obtained after fruit juice production (FJW). Details about cultivation conditions are described in reference (Dmitrović et al., 2022).

Table 1. Quality parameters of the food industry effluents used as media basis for production of microbial biocontrol agents

Parameter/Effluent	pH Value	Dry Matter/Water Content (%, w/v)	Sugar Content (g/L)
CW	4.90	5.81/94.19	50.28^{L}
WFW	3.60	17.01/82.99	19.12^{F} 22.11^{G}
FJW	4.85	0.5/99.5	2.32^{F} 3.31^{G}

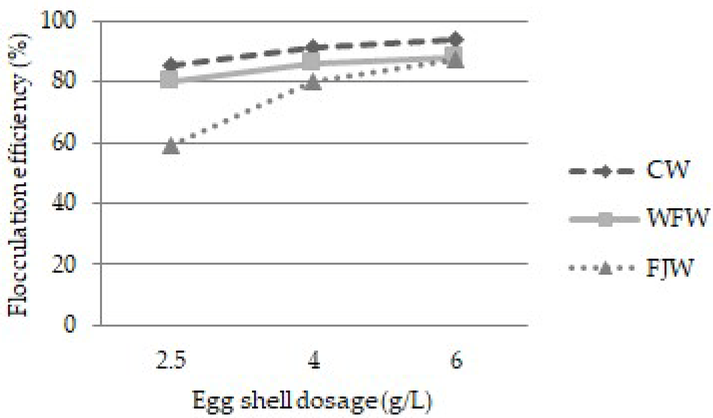
Flocculation test. The jar tests were applied to determine the optimal flocculant dosage. The collected egg shells were cleaned with distilled water and heated at 105°C for 30 minutes to thoroughly evaporate the water content. After that, the dried egg shells were crushed into a fine powder and sieved through a sieve with a mesh size of 200. Finally, the egg shell powder was dissolved in 0.1 M HCl solution and agitated with a magnetic stirrer for 30 minutes. Flocculation assays were conducted in 400 mL glass beakers with 250 mL of the *Bacillus* sp. BioSol021 cultivation broth, obtained after the 96 h bioreactor cultivation to determine the separation efficiency. Following the addition of the egg shell powder to the selected dosage (2-6 g/L, with an increment of 2 g/L), pH was adjusted by dropwise addition of 0.1 M NaOH/0.1 M HCl to a final pH of 7.0. After that, the sample beaker was placed on a magnetic stirrer, first for 5 min of rapid mixing (300 rpm) to disperse the flocculant, and then, the speed was reduced to a slow mixing speed (50 rpm) for 30 min to enhance particle collisions. All flocculation experiments were conducted at the same temperature (25°C). The flocculation efficiency, after 60 min of settling, was calculated by the spectrophotometric measurements of the microbial suspension optical density (absorbance at 600 nm, UV 1800, Shimadzu, Kyoto, Japan) as follows: $\%EF=((OD_0-OD)/OD_0)\times100$**;** where OD_0 and OD are the optical densities of the samples taken before and after the flocculation, respectively.

Antimicrobial activity. After the flocculation tests, antimicrobial activity of the precipitate against the phytopathogen *Aspergillus flavus* strain SA2B SS was examined in vitro using the well diffusion method. In 90 mm Petri plates, a mixture of the SMA medium (15 mL) and fungal pathogen suspension in sterile saline (1 mL–10^5 CFU/mL), homogenized using the vortex mixer, was spread. After the medium solidification, three wells per plate with a diameter of 10 mm were made. In each well, 100 µL of the flocculated sample was added. The incubation was performed at 26°C for 96 h, followed by measurements of the inhibition zone diameters.

Results and discussion

The flocculation efficiency increases with rising egg shell dosage for all effluents used for cultivation. These results are in agreement with the egg shell flocculation for microalgal-bacterial biomass harvesting (Hadiyanto et al., 2021; Choi, 2015). To achieve the necessary flocculation efficiency, a high concentration of nutrient medium requires relatively high flocculant dosage (Yang et al., 2014). As it could be seen in Graph 1, the maximum values of flocculation efficiency in case of cheese whey effluent (94.02%), winery flotation effluent (87.77%) and fruit juice effluent (87.25%) were achieved with an egg shell dosage of 6 g/L.

In this investigation, lower flocculation efficiency was achieved in the case of broth based on the winery and fruit juice wastewater. The reason for this can be found in the fact that dry matter content of winery flotation effluent is significantly higher and dry matter content of the fruit juice wastewater is much lower compared to the cheese whey effluent, which affects flocculation process (Dmitrović et al., 2022).

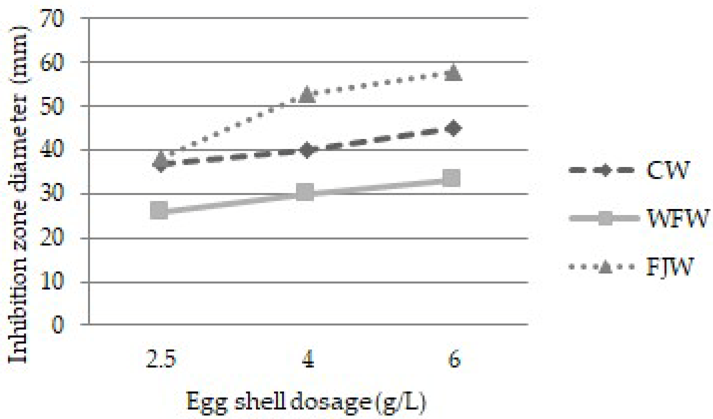


Graph 1. Flocculation efficiency of *Bacillus* sp. BioSol021 with different egg shell doses

Antimicrobial activity of the cultivation broth samples obtained after the flocculation of *Bacillus* sp. BioSol021 from different complex media based on cheese whey wastewater, winery flotation wastewater and fruit juice production wastewater were tested against phytopathogenic isolate of *Aspergillus flavus* SA2B SS.

The effects of the egg shell dosage on the inhibition zone diameter against *Aspergillus flavus* SA2B SS of flocculated broths for all three effluents used for cultivation are shown in Graph 2. In the current study, all cultivation broths were found to have a positive inluence on the bactericidal activity against *Aspergillus flavus*.

The antimicrobial activity of unflocculated broth samples is 42 mm, 35 mm and 60 mm, for broth cultivated on cheese whey effluent, winery flotation effluent and fruit juice effluent, respectively. The inhibition zone diameters after flocculation assays, appears to have maximum values (CW 45 mm, WFW 33 mm, FJW 58 mm) when the egg shell dosage is 6 g/L for all effluent types. The reason for this behaviour can be found in the fact that fermentation suspensions cultivated on wastewater effluents are of complex nature. In addition to microbial cells, cultivation broth also includes a variety of extracellular components and leftover medium nutrients. Some of these extracellular compounds have antimicrobial properties that contribute to the antimicrobial activity of the flocculated samples (Dmitrović et al., 2023).



Graph 2. Inhibition zone diameters of *Bacillus* sp. BioSol021 after flocculation using egg shells against *Aspergillus flavus* SA2B SS

Conclusion

Egg shells were found to be an effective natural flocculant for harvesting *Bacillus* sp. BioSol021 biomass from broth after biological treatment of wastewaters from the dairy, wine and fruit juice industries (cheese whey, winery flotation and fruit juice production wastewaters). It was found that flocculation efficiency up to 94,02% could be achieved with an egg shell dosage of 6 g/L for broth grown on cheese whey effluent. On the other hand, flocculation efficiency of winery flotation wastewater and fruit juice production wastewaters were 87.77% and 87.25%, respectively for the egg shell dosage of 6 g/L. Inhibition zone diameter against *Aspergillus flavus* shows a good viability of

Bacillus sp. BioSol021 cells after egg shell flocculation, but also of the antimicrobial activity of metabolites present in the broth.

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STRATEGIC PLANNING OF TOBACCO PRODUCTION IN MACEDONIA TROUGH A SWOT ANALISIS

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Abstract: The production of oriental tobacco in the Republic of North Macedonia has real development opportunities during a long-term period, viewed from the perspective of world requirements for the representation of oriental types of tobacco in the tobacco mixture in the production of quality cigarettes, as well as for other, broader needs of this type. tobacco at the world level. In addition, precisely the production of tobacco in Macedonia, for a large part of the population provides a condition for improving its social and economic position, engagement of almost all members of the family, creation of work habits (of course, within the framework of non-abuse of child labor) among young people , and thus their possible turning away from bad habits and from reaching out to vices, as well as generally speaking, improving the financial benefits for the state. In perspective, tobacco production, with some oscillations, has a stable trend of movements, with slight upward trends in world production, which we need to adjust and follow.

Bearing in mind the significance and importance of tobacco as an industrial culture from an economic and social aspect, it is necessary to analyze all aspects significant for this sphere, i.e. all weaknesses, strengths, opportunities and threats, sublimated in a detailed SWOT analysis through which perceived the impact of the internal and external environment, as well as the challenges that follow in the future. Precisely because of this, this paper is aimed at analyzing the factors important for tobacco production, by giving recommendations for taking future steps that will lead to greater efficiency and effectiveness of all stakeholders involved in this activity.

Keywords: tobacco production, quality, competitiveness, stable prices, experience, tradition

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Introduction

Macedonia, both regionally and globally, on world stock exchanges and markets, is considered a producer of high-quality aromatic oriental tobacco. The production and trade of tobacco and tobacco products have great economic and social importance, with a share of 3.8% in the gross domestic product for the period 2021/2022. Tobacco is represented by a high 80% of the total area under industrial crops and is one of the most represented exported agricultural products, with 18% participation in domestic exports. The demand for Macedonian oriental tobacco (which is also found in neighboring countries: Bulgaria, Greece and Turkey, but not of the same quality as Macedonian) is still high. Tobacco production as an agricultural branch acquires strategic importance within the state economy, as a significant item of the realized income directly flows into the state budget.

Tobacco is an extremely important crop due to its great influence on the global political, economic and social life of the world's population. But the production of tobacco in the Balkans, in the last five years, is rapidly declining. And in Greece, the production of tobacco has been drastically reduced, because as a member of the European Union, in recent years, high subsidies were available for tobacco production in order to reorient the tobacco producers to the cultivation of some other crops. In Bulgaria, tobacco production has also decreased because, as a member of the EU, the country was called upon to reduce its tobacco production areas (tobacco production quota fell in relation to its production even before becoming a member of the EU). In Serbia, the production of oriental tobacco is almost eliminated, and only Virginia and Burley tobacco are produced (5,000 to 6,000 tons on average). Albania, as a producer of oriental tobacco, has a symbolic share of about 2,000 tons per year.[2] Macedonia produces only oriental tobacco. Production oscillates from 16,000 tons to 26,000 tons in the past ten years. It has stable production, with high quality tobacco, well known to the world's largest tobacco companies.[3]

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 on the trends of modern world achievements in that area. The preferred standards of future development point to an increasing focus on production, both on its quantitative limitation and on

[2] Food and Agriculture Organization of the United Nations - FAO

[3] National strategy for agriculture and rural development for the period 2021-2027, Ministry of Agriculture, Forestry and Water Management of the Republic of North Macedonia

its qualitative worthiness, environmental protection, social security, economic efficiency, the healthy way of growing and developing plants, as well as compliance with the guidelines of the World Health Organization. In that direction, it is extremely important to make a special analysis with an appropriate methodology that will indicate all the opportunities and advantages that are available to tobacco production in Macedonia, but also to emphasize the weaknesses and future threats that can affect the reduction of production and its replacement with other crops. For those reasons, using the SWOT analysis and its methodology can greatly contribute to the strategic planning of tobacco production in Macedonia.

Material and method

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 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 the use of the SWOT analysis to accept strengths, target opportunities and overcome weaknesses and threats.

Results and discussion

Good climatic and soil conditions are one of the primary factors for tobacco production in Macedonia. Tobacco is grown on sandy soils of poor quality, where no other crop shows such good and profitable results for the producers. It tolerates wide variations in climatic conditions, but still achieves respectable yields. Oriental types of tobacco are often grown on poorer soils, for which the climatic conditions in most regions of Macedonia appear to be very suitable. There are different types of tobacco that are compatible with the climatic differences of the country and with the different regions in which they are grown. For each oriental type of tobacco produced in Macedonia (Prilep, Jebel and Jaka) common characteristics are their quality and specific aroma.

The fact that tobacco is a relatively perishable product compared to other agricultural products, as well as the fact that it is relatively easy to store and transport, make tobacco cultivation desirable. The still high level of unemployment largely contributes to this (according to the State Statistics Office of the Republic of Macedonia, in the fourth quarter of 2021, unemployment is 15.2%), as well as the fact that, especially in rural regions, tobacco represents a means of livelihood.[4] Although the unemployment rate has been decreasing in recent years, it is still very high, at least in rural areas. Thus, when we say that tobacco is a culture that provides livelihood for a certain population, we especially want to point out that Macedonia has a relatively cheap labor force, which indicates the possibility of obtaining lower prices for products, and thus greater market competitiveness.

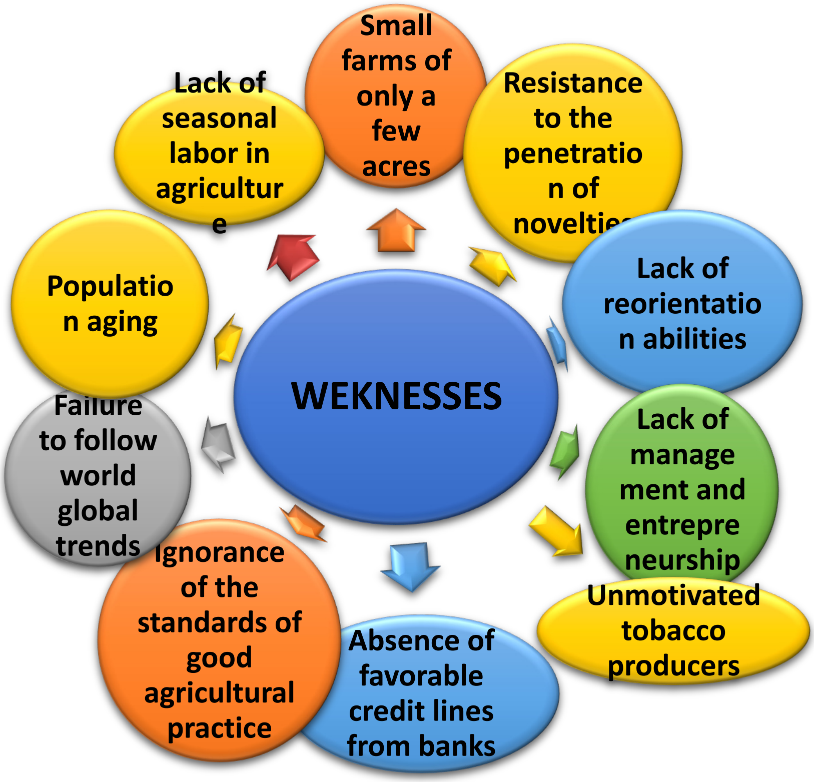
Macedonia has experienced tobacco producers, some of whom have been growing tobacco for several generations, as well as tobacco experts educated in agricultural schools (secondary schools, colleges and institutes). Of particular importance in this domain is the functioning of the Scientific Institute for Tobacco - Prilep, which employs professional, high-quality, capable and specialized personnel, who work on developing and controlling the process of tobacco production, on creating new tobacco varieties, protection from tobacco diseases, production of certified tobacco seed, as well as additional education and assistance for each tobacco grower. The advantage for tobacco producers is that there is a guaranteed purchase for the tobacco, as well as a certain amount of subsidies which, in recent years, depend on the quality of the delivered tobacco, that is, on the class. Since it is a relatively demanded product not only in Macedonia but also on the world market, a part of the production of oriental products is intended for export, which creates a significant inflow of foreign currency in the country. In general, tobacco exports are intended for countries such as the United States, Greece, Germany, Switzerland and others. In Macedonia, there is a well-defined legal regulation that is constantly being worked on in order to meet the needs of individual tobacco producers, but at the same time to protect them. One of the particularly important positives is the existence of a well-developed network of primary producers, tobacco buying firms and cigarette processors. All producers are protected by the existence and conclusion of a contract for the purchase of tobacco, which makes this crop relatively desirable for production. The existence of multiple buying companies creates a good competitive climate. The relatively stable price and the existence of additional direct payments by the state make this product even more

[4] State Statistics Office of the Republic of North Macedonia

competitive than other products. These advantages of tobacco production in Macedonia are shown by Scheme 1.

Scheme 1 Advantages of tobacco production in Macedonia

If we count traditionality and experience among the strong points of tobacco production and cultivation, that very traditionality is also a weak point of tobacco production. Tradition is one of the characteristics that binds the people living in the villages to their fields and to the traditional way of growing tobacco. Their lack of additional skills leaves them with no choice and makes them addicted to tobacco cultivation even when the purchase prices are relatively low, ie. is. when bad market conditions exist. The absence of skills for management, reorientation, production planning, makes these tobacco producers even more vulnerable. This shows that there is no visible strategy that would allow tobacco producers to have valuable information about the market before planning production or how to improve product quality and thereby secure better prices. Traditionality is also related to other elements. Thus, it is mostly a question of small farms or family businesses of only a few acres. Such small farms in Macedonia have big problems in achieving low production costs and applying more mechanization in the production process. Macedonian tobacco producers are mostly small landowners who have little opportunities to expand their land due to the undefined structure of the land market and the poor allocation of state agricultural land for rent. Poorly developed relationships between producers and buyers are caused by many factors. Tobacco buying companies do not always fulfill their obligations defined in the contract. The delay in the beginning of the purchase of raw tobacco in certain years, the lower prices given to the producers at the buying points and the delay in payment, are just a few problems that shake the confidence between the producers and the buyers. All these problems reduce the interest in tobacco production – a fact that is reflected and can be seen by the decrease in the areas planted with tobacco, analyzed from 2010 until now. Demographic structure is another weakness of tobacco production. The Macedonian population, which is engaged in agriculture, and therefore in tobacco production, is getting older. Young people see their livelihood in cultivating the land less and less, so if steps are not taken that would restore the interest of the young population to tobacco cultivation, after a few decades there will not be enough labor to cultivate the already existing areas, and, of course, the probability of its increase decreases. Here it is important to emphasize the fact that the existing workforce in tobacco production does not have a sufficient level of education, which contributes to the absence of the desire to cooperate and gain experience in using the appropriate funds. All these weaknesses are represented by Scheme 2.



Scheme 2 Weaknesses of tobacco production in Macedonia

One of the main opportunities that must be intensively worked on, especially in the coming period, is the possibility of utilizing the uncultivated agricultural land that is in state ownership. A second possibility that should be paid attention to is the reduction of the fragmentation of the already existing tobacco production entities, in order to improve their efficiency and effectiveness. Education of tobacco producers, which would encourage tobacco production, but also raise it to the level of a family business, is the third possibility that should be considered, especially through the monitoring of seminars, trainings, debates, as well as the increasing involvement of the expert public and bringing it closer to the problems on the ground. The introduction of production quotas for tobacco in the member states of the European Union, as well as the reduction of production in these countries due to the lack of interest of producers to engage in this type of business, means for Macedonia the creation of new opportunities for the expansion of production, especially in a period when stagnation of the production of oriental types of tobacco, not only in our immediate neighborhood but also more widely, in the world. As an opportunity for future and current tobacco producers, the idea and plan for additional production should be developed, which would achieve better utilization of production factors. In order to improve and increase tobacco production, it is necessary to enable cheap credit lines, which would create easy access to fresh capital that will be used both for the modernization of outdated equipment and for the expansion of production capacities, i.e. for the cultivation of larger areas. The previously mentioned possibilities are schematically presented in the attachment.

Scheme 3 Opportunities of tobacco production in Macedonia

In addition to the opportunities for development, threats to tobacco production are also present (Scheme 4). The first and biggest threat is that, after Macedonia's entry into the European Union, we are expected to receive reduced production quotas, imposed by the need to comply with the existing CAP (Common Agricultural Policy) which does not foresee the existence of direct payments for tobacco producers, but payments after area or rural development payments. This kind of agrarian policy at this moment corresponds very little to our agrarian policy. The next threat is the danger of reducing the participation of oriental types of tobacco in modern blend cigarettes, as well as the increasingly frequent use of electronic tobacco products. A third threat is the danger of increasing taxes in order to fill the budgets of the countries due to the existing crisis, and thus an increase in the price of cigarettes, as well as the increasingly present aggressive campaign against smoking. Today, tobacco is considered a great threat to people's health and, against it, i.e. against smoking, very aggressive measures are proposed. The decline in smoking is beginning to affect the demand for tobacco and the quantities of tobacco produced in Europe and around the world. The drop in the price of tobacco at the world level, the use of obsolete equipment, as well as globalization, the monopolization of the tobacco market and the market of tobacco products (cigarettes) by a small number of companies, are the dangers that further threaten this species. production. However, the greatest responsibility should be borne by the Government, during the pre-accession negotiations for entry into the European Union, because without the provision of respective quotas for tobacco production, all previous efforts would be ineffective.

Scheme 4 Threats to tobacco production in Macedonia

This delineation of all aspects and opportunities for the development of tobacco production, through the use of SWOT analysis, gives good results in terms of taking future measures and steps. The application of this analysis in practice proves to be very effective, in every area. Its history begins in 1960, at the Stanford Research Institute in America, where Albert S. Humphrey and his team created a matrix for identifying and analyzing the strengths and weaknesses, opportunities and threats of a company. The main goal was to increase awareness of the factors that contribute to making a decision or establishing a strategy. Initially, the matrix was named SOFT (Satisfactory, Opportunity, Fault, Threat), so that later the renaming to SWOT proved to be more reliable for use in the various departments in the organization, but also to be applied personally for oneself, for self-knowledge, for making a decision and achieving the intended goal. Before starting the application, of course, the most important thing is to define the goal and devise a way or a strategy how to start to reach its achievement.

In short, the SWOT analysis is an acronym for Strengths, Weaknesses, Opportunities, Threats (strengths, weaknesses, opportunities, threats), where the first two listed characteristics are internal factors, and the remaining two are external factors. With the help of this analysis or matrix, it is much easier to make a decision about the next steps that we plan and it creates a visual representation of the current moment in which we are. This type of analysis, although it is mostly intended and used in companies and has a very important role, can be very simply applied in the management of self-development.

Conclusion

The research and analysis done through the SWOT method for comparing the weaknesses, advantages, opportunities and threats, specifically in the sphere of tobacco production in Macedonia, indicates that in order to achieve greater efficiency and effectiveness in production and create conditions for prosperity in the future, emphasis should be placed on the following parameters:

- Perception and understanding of modern world achievements in the field of management and organization of tobacco production;

– Continuous improvement of processes based on objective measurements and analyses;

- Creation of results in the course of work, through expressed motivation and satisfaction during work, which result, first of all, from proper management;

- Taking care of the continuous improvement of the overall quality in the operation;

– Establishing a system for monitoring the achievement of set goals through monitoring, data analysis, internal checks, etc.

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BENTHIC ALGAE AS BIOINDICATORS IN ASSESSING ECOLOGICAL STATUS OF ČEMERNICA AND DIČINA RIVERS

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Abstract: This study aimed to assess the ecological status/potential of the Čemernica and Dičina rivers (Zapadna Morava basin) based on epilithic diatoms and supporting physico-chemical parameters, as well as to explore indicative properties of the detected macroalgae. The sampling and analysis of benthic algae and measurement of physico-chemical parameters were carried out in August 2023. The ecological status/potential of the Čemernica River was poor to bad at all investigated sites (ČR1, ČR2, ČR3) based on diatom indices and physico-chemical parameters. Low diatom indices values and the presence of the macroalgae *Stigeoclonium tenue* indicated organic pollution. In the Dičina River, good ecological status was assessed at all sites (DR1-DR4) based on the diatom indices, while poor ecological status has been assessed at almost all sites (DR1, DR2, DR3) based on the physico-chemical parameters, except at DR4, where the ecological status was good. The high coverage of *Cladophora glomerata* in both rivers indicated nutrient loading, which aligned with the results of the physico-chemical parameters.

Keywords: water quality, diatom indices, macroalgae

Introduction

The Čemernica River and its longest left tributary, the Dičina River, are hilly-mountain rivers in southwestern Serbia within the Zapadna Morava River basin. The lower reaches of both rivers are indirectly polluted by industrial wastewater from Gornji Milanovac, which has led to several fish mortality events in the last two decades. In general, there is a lack of water quality studies of these rivers. The Čemernica River has only been investigated by Jurišić (2004), who explored the diversity of benthic algae and water saprobity. The monitoring program of the Serbian Environmental Protection Agency (SEPA)

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includes two sites on the Čemernica River and one site on the Dičina River. In addition, the Public Health Institute Čačak conducts monthly measurements of physico-chemical parameters at a single site on the Čemernica River.

The aim of this study was to assess the ecological status/potential of the Čemernica and Dičina rivers in accordance with the WFD (2000) (*Water Framework Directive*) based on the phytobenthos (epilithic diatoms) and supporting physico-chemical parameters, as well as to explore indicative properties of detected macroalgae.

Materials and methods

The field research was carried out in August 2023 and included three sites on the Čemernica River: ČR1 (43⁰58′55.3'' N, 20⁰15′51.1'' E, altitude 308 m), ČR2 (43⁰55′39.9'' N, 20⁰19′29.8'' E, alt. 260 m), ČR3 (43⁰54′45.2'' N, 20⁰24′12.8'' E, alt. 234 m) and four sites on the Dičina River: DR1 (44°06'15.4'' N, 20°15'54.2'' E, alt. 485 m), DR2 (44°03'44.6'' N, 20°18'49.7'' E, alt. 385 m), DR3 (44°02'43.6'' N, 20°22'15.8'' E, alt. 350 m), DR4 (44°01'31.8'' N, 20°21'30.9'' E, alt. 339 m).

The sampling of benthic algae, their qualitative and quantitative analysis, the measurement of physico-chemical parameters and ecological status assessment were carried out using the equipment, standard methods and regulations described in the study by Simić et al. (2018).

Results and discussion

During the research in August 2023, a total of 46 algal taxa were recorded in the Čemernica River: Cyanobacteria (1), Bacillariophyta (38), Chlorophyta (4), Charophyta (2), and Euglenophyta (1). A total of 35 taxa were recorded in the Dičina River: Cyanobacteria (1), Rhodophyta (2), Heterokontophyta (1), Bacillariophyta (27), Chlorophyta (2), Charophyta (1), and Euglenophyta (1).

The results of the quantitative analysis of the epilithic diatoms at the investigated sites are presented in Table 1.

Table 1. Quantitative analysis of epilithic diatoms in the Čemernica (ČR1, ČR2, ČR3) and Dičina (DR1, DR2, DR3, DR4) rivers - August 2023

Taxa / Site	**ČR1**	**ČR2**	**ČR3**	**DR1**	**DR2**	**DR3**	**DR4**
	%						
Achnanthidium minutissimum (Kütz.) Czarn.							2.75
Achnanthidium sp.	2.25						

Amphora ovalis (Kütz.) Kütz.	0.25	1	5.75			4.5	3.5
Amphora sp.			2				0.75
Cocconeis pediculus Ehrenb.	1	11.25	2.5		**76.5**	12.75	8
Cocconeis placentula Ehrenb.	11	1	3	**67.6**	3.5	10.5	3
Craticula cuspidata (Kütz.) Mann			0.5				
Cymbella affinis Kütz.	15	14.5	0.25	4		0.25	1
Cymbella aspera (Ehrenb.) Cleve	2	2.25		1.6			0.75
Cymbella compacta Østrup		0.5					
Cymbella neocistula Krammer	0.75	1.75					
Cymbella perparva Krammer		0.75					
Cymbella subcistula Krammer	0.25						
Cymbella tumida (Bréb. ex Kütz.) Van Heurck			0.5				
Diatoma vulgaris Bory	0.25	1	1.25	8		0.5	3.75
Encyonema leibleinii (Aga.) Silva							1.75
Encyonema minutum (Hil.) Mann	3.75	6.25	0.75	4			0.25
Encyonopsis minuta Krammer	1	0.25					
Gomphonema truncatum Ehrenb.	0.25					0.75	
Gyrosigma acuminatum (Kütz.) Rabenh.				0.8			1
Gyrosigma attenuatum (Kütz.) Rabenh.			1.75				0.75
Melosira varians Aga.			**46.5**	1.6	9	**35**	**44**
Navicula capitoradiata Germ. ex Gasse						13.25	12
Navicula cryptocephaloides Hust.			3.25				
Navicula radiosa Kütz.	0.75		0.75				
Navicula sp.						0.5	
Navicula subrhynchocephala Hust.	1.75	1.25					
Navicula tripunctata (Müll.) Bory	0.75		1.5			11.75	14
Navicula viridula (Kütz.) Ehrenb.			2.5			2.5	0.25
Nitzschia brevissima Grunow		0.5					
Nitzschia communis Rabenh.	0.75						
Nitzschia denticula Grunow	1.75	1.25					
Nitzschia dissipata (Kütz.) Rabenh.	1.75						
Nitzschia linearis (Aga.) Smith	1.5	0.25	0.25		7.5	1	0.75
Nitzschia palea (Kütz.) Smith	**43.25**	**42.75**	13				
Nitzschia sp.							0.25
Rhoicosphenia abbreviata (Aga.) Lange-Bert.	4	0.5	5	12.4	2	4.5	0.5

Stauroneis kriegeri Patrick						0.25	
Stauroneis sp.	0.25						
Surirella lacrimula English			0.25				
Surirella librile (Ehrenb.) Ehrenb.			4.5				
Surirella tenera Gregory			1.25			1	
Surirella undulata Ehrenb.			0.25			0.25	0.25
Ulnaria ulna (Nitzsch) Compère	5.75	13	0.75		0.5	0.75	0.75
Ulnaria ulna var. *contracta* (Østrup) Morales			0.75		1		

The following benthic macroalgae were detected: *Cladophora glomerata* (L.) Kütz. (ČR1- 95%; ČR2- 10%; ČR3- 30%; DR2- 40%; DR3- 90%; DR4- 40%), *Stigeoclonium tenue* (Agardh) Kütz. (ČR1- 1%), *Hildenbrandia rivularis* (Liebmann) Agardh (DR1- 10%), *Audouinella pygmaea* (Kütz.) Weber Bosse (DR2 and DR3- 1%), *Microcoleus autumnalis* (Gomont) Strunecký et al. (DR3- 1%), and *Tribonema regulare* Pascher (DR4- 5%). The physico-chemical parameters measured at the sites of the Čemernica and Dičina rivers are listed in Table 2.

Table 2. Measured physico-chemical parameters at the investigated sites of Čemernica (ČR1, ČR2, ČR3) and Dičina (DR1, DR2, DR3, DR4) rivers

Parameter / Site	**ČR1**	**ĆR2**	**ČR3**	**DR1**	**DR2**	**DR3**	**DR4**
pH	7.9	8.12	8.11	7.4	6.7	6.7	7.5
Oxygen conc. (mg/L)	7.67	8.79	8.76	9.46	13.10	9.56	11.69
BOD (mg/L)	1.9	3.8	4.25	1.3	1.9	1.25	1.95
PO_4-P (mg/L)	0.36	0.58	1.18	0.16	0.38	0.47	0.09
TP (mg/L)	0.12	0.19	0.38	0.05	0.12	0.15	0.03
NO_3-N (mg/L)	<4	12	14	9	8	8	<4
NH_4-N (mg/L)	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03

Based on the diatom indices, the ecological status of the Čemernica River was assessed as bad at ČR1 (IPS=7; CEE=5.6) and ČR2 (IPS=7.2; CEE=4.8), while poor ecological potential was assessed at ČR3 (IPS=10.9). At all sites of the Dičina River, the ecological status was assessed as good based on the diatom indices (IPS=15.5, CEE=13.8; IPS=14.8, CEE=12.2; IPS=14.2, CEE=12.2; IPS=14.6, CEE=12.6; respectively). Considering that the IPS and CEE indices are primarily indicative of organic pollution (Cemagref, 1982; Descy and Coste, 1991), it can be concluded that the Čemernica River, unlike the Dičina River, was impacted by organic matter. Based on physico-chemical parameters, a poor (ČR1) to bad (ČR2) ecological status and a bad ecological potential (ČR3) were assessed along the Čemernica River, while the Dičina River sites exhibited a good (DR4) to poor (DR1, DR2, DR3) ecological status. Jurišić (2004) determined that the water of the Čemernica River is β- to α-mesosaprobic based on the presence of benthic algae. SEPA monitoring in 2012/13 determined moderate ecological potential for ČR2 and poor ecological status for ČR3 based on physico-chemical parameters, while both sites exhibited moderate ecological status/potential based on diatom indices (Denić et al., 2015). The results of the Public Health Institute Čačak in August 2023 show a moderate ecological status based on physico-chemical parameters (Anonymous, 2023). Our results suggest a deterioration in water quality compared to all previous studies.

Although macroalgae were not used in the assessment of ecological status in Serbia, their usage is recommended by the WFD (2000) due to their indicative properties. The green alga *Cladophora glomerata* tends to cover entire riverbeds at high nutrient concentrations (Michalak and Messyasz, 2020), which was observed in ČR1 and DR3. *Stigeoclonium tenue*, detected in ČR1, is known to indicate organic pollution (Jafari and Gunale, 2006) and is associated with poor to bad water quality (Carmoma-Jimenez et al., 2022), which is consistent with our results. The red alga *Hildenbrandia rivularis*, previously known as an indicator of oligotrophic waters, is considered a species resistant to inorganic pollution according to recent data (Jakubas-Krzak et al., 2023). This is confirmed by the discovery of this species at the DR1 site, where high nitrate concentrations were detected.

Conclusion

The Čemernica River exhibits a poor to bad ecological status/potential at all surveyed sites (ČR1, ČR2, ČR3), based on diatom indices and physico-chemical parameters. At all sites of the Dičina River, based on the diatom indices, good ecological status was assessed, while based on physico-chemical parameters it ranged from good (DR4) to poor (DR1, DR2, DR3). Some of the identified species of macroalgae have proven to be good indicators of inorganic and organic pollution in these rivers.

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INFLUENCE OF DIFFERENT SOIL USE ON VERTISOL WATER INFILTRATION

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Abstract: On Vertisol soil type, in the area of Bela Crkva, within the cadastral municipality of Crvena Crkva, research on the impact of different land uses on infiltration rate was conducted in 2021. Infiltration tests were carried out using double metal cylindrical infiltrometers on plots under shrubs, meadows, and cultivated land. It was found that the infiltration rate was highest on the pasture plot compared to the cultivated land and shrubs, which can be attributed to the characteristics of Vertisol and the developed root system that permeates the soil. The obtained results should be taken with caution, considering that the soil is a polydisperse system, and it can contribute to predicting surface runoff, assessing Vertisol waterlogging, and choosing irrigation methods.

Keywords: land use, infiltration capacity, total infiltration

Introduction

In the last few decades, land use/coverage has significantly changed due to climate change and human activities. These changes lead to differences in infiltration characteristics between different land uses/coverages (Jarvis et al., 2013; Reichert et al., 2022).

Infiltration represents the ability of dry soil to absorb and allow water to pass through its surface. Quantitatively, it is expressed as the depth of water that enters the soil over its surface per unit of time. This parameter characterizes the first phase of permeability when free pores in the soil gradually fill with water. It lasts until the soil becomes fully saturated with water. The movement of water through saturated soil under the influence of gravity represents the second phase of permeability or filtration (Gajic B., 2005). Infiltration depends on the total amount of pores in the soil, primarily large pores, cracks, and pedofauna burrows. In the case of an unstable structure, the initial infiltration rapidly decreases due to the breakdown of structural aggregates during their

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wetting and swelling of soil colloids. Wu et al., 2019 and Zhu et al., 2020, state that infiltration decreases with increasing soil bulk density. Regarding organic matter content, studies by individual researchers indicate that infiltration increases with increased organic matter content (Fu et al., 2017; Zhu et al., 2020). However, opposing views are also mentioned, especially in soils with low organic matter content or under drought conditions (Jarvis et al., 2013; Zhu et al., 2020). Knowledge of the size and nature of infiltration is of great importance for agronomic and meliorative soil assessment. The data on this parameter are used to determine surface runoff, irrigation norms, etc. The maximum infiltration rate at which water enters the soil - infiltration capacity, is a dynamic parameter that varies throughout the year depending on the soil profile's permeability, the condition, and characteristics of the soil surface, and the amount of water in it. Therefore, when analyzing registered infiltration test data, deviations can be observed within one location. Generally, soils with high clay content are prone to consolidation, resulting in poor soil structure, low porosity, and high bulk density (Wang et al., 2023; Zhu et al., 2020). The higher the soil moisture content, the lower the infiltration rate. The size of infiltration varies seasonally, and variations also exist from one testing location to another, so it is necessary to perform multiple tests to obtain real data on average infiltration. It also depends on the current condition and previous land use of the plot, as well as the application of agrotechnical measures. Soil cultivation can have a significant impact on infiltration (Cameira et al., 2003). Previous studies (Bormann & Klaassen, 2008; Lopes et al., 2020) have confirmed that infiltration increases after the conversion of intensively used land. Improper human use leads to soil degradation (Bhushan & Yadav, 2000; Gajić et al., 2017). Changing land use, such as clearing natural pastures to increase arable land or replacing degraded arable land due to long-term improper use, inevitably leads to changes in the physical properties and characteristics of pedogenetic processes in the soil. Such land conversion and clearing can disrupt the hydrological balance of the area, increasing the risk of erosion and floods on one hand, and intensifying soil drought, which can result in soil degradation and the absence of expected economic benefits. Water infiltration is a dynamic process that varies over time and space and plays an important role in replenishing soil water reserves, which are crucial for optimal crop growth and development. This study aims to determine the impact of different land uses of the investigated Vertisol site on its infiltration characteristics, predict the characteristics of water movement and dissolved substances in the soil profile, and potentially suggest a choice of irrigation method.

Material and Methods

The study location included the investigation of areas under shrubs (N:529568; E:4973808), grass vegetation - pasture (N:529668; E:4974131), and cultivated land (N:529250; E:4974714) in the area of Bela Crkva within the cadastral municipality of Crvena Crkva.

Fieldwork included sampling soil from the surface horizon using standard sampling methods to a depth of 30 cm in disturbed and undisturbed conditions.

Soil infiltration properties were measured using a double-ring infiltrometer with an inner and outer diameter of 30 cm and 60 cm, respectively, in three repetitions for 200 minutes until a steady infiltration rate was reached.

Sample preparation in disturbed conditions was conducted through the process of drying, grinding, and sieving. Analyses were conducted by applying standard test methods and methods whose source is in professional literature (JDPZ, 1966; JDPZ, 1997). Statistical methods were applied for data processing using Excel.

Results and Discussion

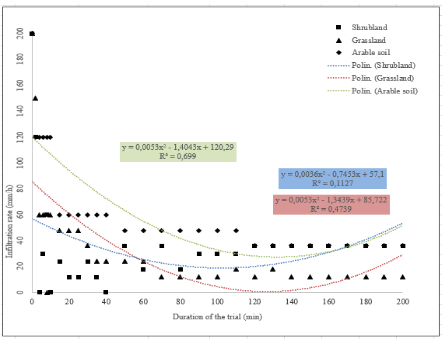
In the area of the cadastral municipality of Crvena Crkva, Vertisols (WRB, 2015) were formed on a loess-like substrate that is composed of light clay, with a slightly lighter mechanical composition compared to typical Vertisols on lake sediments. They are characterized by a very deep humus-rich Avt horizon, thicker than 100 cm, which provides good conditions for plant rooting, water retention, and nutrient availability. However, this type of soil is characterized by unfavorable water-air and thermal regimes due to the unfavorable structure, both in terms of form and size of aggregates, as well as their stability. The aggregate composition changes with changes in moisture conditions - in dry conditions, the soil becomes compact and cracks, while when saturated with water, it softens and swells, with poor water permeability, which negatively affects porosity and aeration. The limitation of this type of soil is reflected in the short interval for cultivation, which is conditioned by the current moisture content. The tested soil samples in the plow (cultivated) horizon, which was also investigated, belong to the light clay (LC) texture class. The values of soil bulk density at the tested sites are slightly lower in the cultivated area compared to the other two sites, while the values of total porosity are higher as a result of changes in land use. The results of the analyzed soil samples are presented in Table 1.

Table 1. Physical and chemical properties of the tested soil samples.

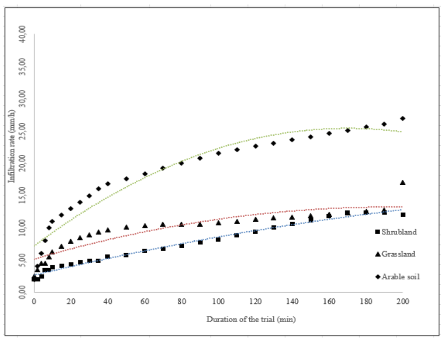
Land usage	Partical density (g/cm^3)	Bulk density (g/cm^3)	Porosity (%)	Humus (%)	Fractions			Texture (IUSS, 2014)
					Total sand (2,00-0,02 mm)	Silt (0,02-0,002	Clay (<0,002 mm)	
Shrubland	2,71	1,35	50,18	4,48	35,70	28,80	35,50	LC
Grassland	2,70	1,35	50,00	4,05	35,10	29,80	35,10	LC
Arable land	2,76	1,33	51,81	3,47	36,40	28,90	34,70	LC

*LC -Light Clay

In the tested samples, the organic matter content at the Arable soil site is lower compared to the Shrubland and Grassland sites, but still within the range of moderate provision. Figure 1 shows the water infiltration rate curves in the soil. It can be observed that the initial infiltration rate after 2 minutes of measurement was highest in the pasture vegetation plot (150 mm h^{-1}), while in the Shrubland and Arable soil plots, it was 120 mm h^{-1}, which is about 20% lower, likely due to the higher root mass expressed in the pasture site. According to Duiker et al., 2001 and Gajić et al., 2017, when the infiltration rate is higher, runoff is generally lower, which also contributes to soil erosion reduction. The time required to reach a constant infiltration rate differed between plots and varied between 120 minutes for shrubland and arable soil, and 140 minutes for grassland soil.



Graph 1. Average water infiltration rates



Graph 2. Cumulative infiltration

The change in land use had an impact on cumulative infiltration, as shown in Figure 2. Cultivated soil had the highest cumulative infiltration. The difference in initial infiltration and the decrease in infiltration rate over time between the studied treatments indicate a greater ability of the pores in cultivated soil to increase the amount of infiltrated water before filling the micropores and reaching a steady rate. The presence of plant roots in the pasture area likely contributed to higher water infiltration compared to the cultivated plot.

Conclusion

Based on the investigation of infiltration characteristics of Vertisols under different land uses, it was found that in this type of soil, the presence of a grass cover or a deep root system can increase the infiltration rate. The obtained results should be taken with caution due to the polydisperse nature of the soil. The physical, mechanical, and chemical properties of this type of soil, especially the increased clay content, require agromeliorative measures to avoid the formation of a plow pan that occurs due to constant plowing at the same depth, above which water tends to accumulate during wet periods.

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THE INFLUENCE OF SATURATED SOLUTIONS OF MINERAL SALTS ON SAPROPHITIC BACTERIA IN THE SOIL

Dragutin Đukić[1], Leka Mandić[1], Vesna Đurović[1], Monika Stojanova[2]

Abstract: The paper provides an overview of the influence of saturated salt solutions on the number and structure of bacterial complexes in soil, as well as pure cultures of bacteria isolated from that soil. The short-term action of high doses of salt is not fatal for saprophytic bacteria in the soil. Nitrogen salts exert the greatest influence on the mentioned parameters, especially ammonium nitrate. Bacilli and sliding bacteria are the most resistant to the effects of salt.

Keywords: bacteria, mineral salts, soil

Introduction

Application of mineral fertilizers is a very pronounced anthropogenic impact on the biosphere. The introduction of granulated mineral fertilizers into the soil is very common, whereby loci with high concentrations of salt are formed near the granules, which can lead to disturbances in the normal functioning of the living world. Many studies have determined the unique stability of the soil microbial system (Mihnovska, 1982; Pavlenko, 1982; Jemcev, Mišustin, 1993; Jemcev, Đukić, 2000; Đukić et al., 2020), however, based on the data from the existing literature, it cannot be given a clear answer about the reaction of bacteria to the introduction of high concentrations of mineral salts.

The aim of this review is to look at the short-term impact of high salt concentrations on saprophytic bacteria in the soil and determine the specificity of the resulting changes.

The influence of saturated salt solutions on the taxonomic structure of saprophytic bacteria in the soil

The influence of saturated salt solutions $CaHPO_4$, KCl, NH_4NO_3, NH_4Cl and KNO_3 during 24 hours on the taxonomic structure of bacteria in the meadow soil of the flood zone will be presented. The introduction of NH_4NO_3, NH_4Cl and

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KNO_3 into the soil leads to a decrease in the number of saprophytic bacteria by 1.3 to 2.5 times. At the same time, the maximum effect is observed when introducing NH_4NO_3 (Fig.1). The introduction of KCl does not have a significant effect on the number of bacteria, while the introduction of $CaHPO_4$ and $CaCO_3$ slightly increased the number of bacteria in the soil. NH_4NO_3 shows the greatest toxicity to saprophytic bacteria. However, the decrease in abundance by only 2.5 times testifies to the relatively weak effect of short-term introduction of saturated salt solutions on bacteria (Lapigina and Lisak, 2001).

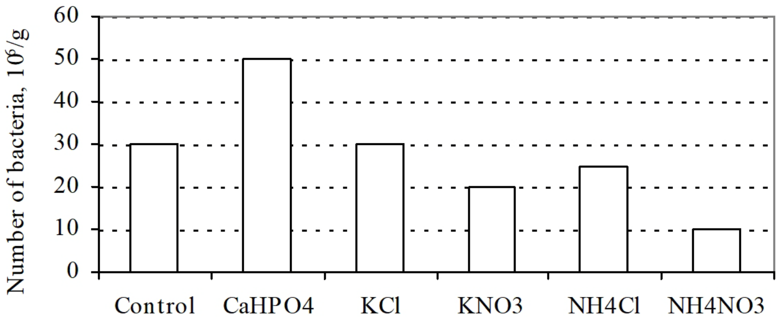

Fig. 1. Change in abundance of saprophytic bacteria in meadow soil under the influence of high doses of mineral fertilizers (Lapigina and Lisak, 2001)

The change in the abundance of saprophytic bacteria is not related to differences in soil acidity (before and after soil treatment with mineral fertilizers). It was shown that the single intake of high doses of salt does not cause significant changes in pH value - the maximum decrease in pH (from 7.7 to 6.4) occurs only with the intake of NH_4NO_3. The introduction of NH_4NO_3 causes significant changes in the structure of the saprotrophic complex of bacteria in the meadow soil (Fig. 2). In the control soil, streptomycetes and gram-negative bacteria dominate, arthrobacteria and bacilli are subdominant, and minor components are rhodococci, micrococci and sliding bacteria, while when NH_4NO_3 is introduced, streptomycetes absolutely dominate (63%), and the subdominant position is occupied by arthrobacteria, bacilli and gram-negative bacteria. The introduction of KNO_3 and NH_4Cl leads to a significant redistribution in the structure of bacteria, while the introduction of $CaHPO_4$ and KCl does not cause significant changes in the taxonomic composition of saprophytic bacteria. With the introduction of salts KCl, NH_4NO_3, NH_4Cl and KNO_3, gram-negative bacteria of the genera *Spirillum*, *Vibrio* and *Pseudomonas* are completely inhibited. Bacteria of the genera *Polyangium*, *Cytophaga* and *Myxococcus* are less sensitive to the effects of salt.

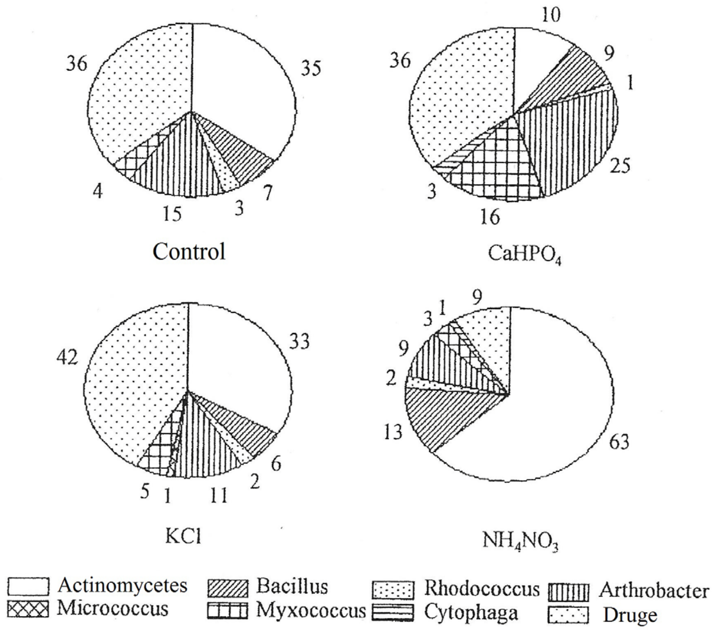

Fig. 2. Changes in the structure of the bacterial complex in meadow soil under the influence of high doses of mineral salts (Lapigina and Lisak, 2001)

Among pure cultures of bacteria isolated from meadow soil (genera *Bacillus, Arthrobacter, Rhodococcus, Polyangium, Cytophaga, Myxococcus*) *Bacillus* are the most resistant to saturated salt solutions, which can obviously be related to their ability to form spores, while arthrobacteria and rhodococci are less resistant (Zhang et al., 2017; Mandić et al., 2022). Among gram-negative bacteria, bacteria of the genera *Myxococcus* and *Polyangium* exhibit significant resistance, which are capable of creating myxospores with high resistance to various unfavorable factors, while representatives of the genus *Cytophaga* are less resistant (Fig. 3).

A comparative analysis of the behaviour of bacteria in pure cultures and directly in the soil during the introduction of mineral salts (Fig. 4) shows that bacteria in the soil are more resistant to salts than pure cultures of bacteria, which is manifested by a less pronounced decrease in their number which is in agreement with the results of other authors (Mandić et al., 2005).

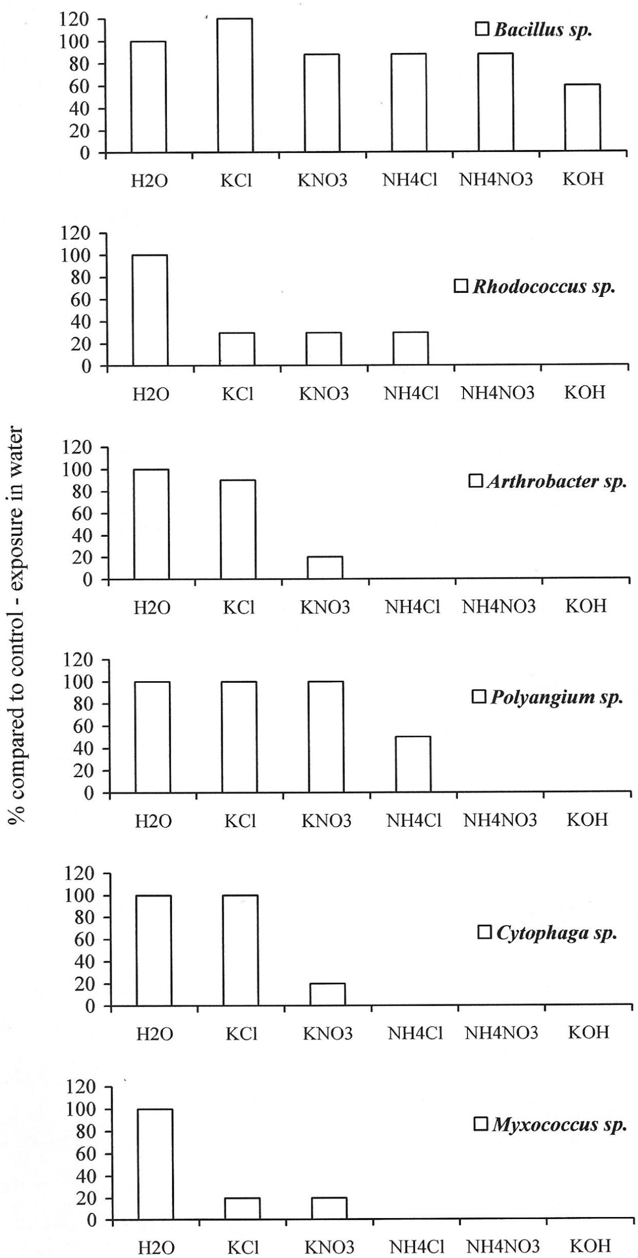

Fig. 3. Effect of short-term exposure (24h) of saturated salt solutions and 1N KOH on survival (in % compared to control - exposure in water) of pure bacterial cultures (Lapigina and Lisak, 2001).

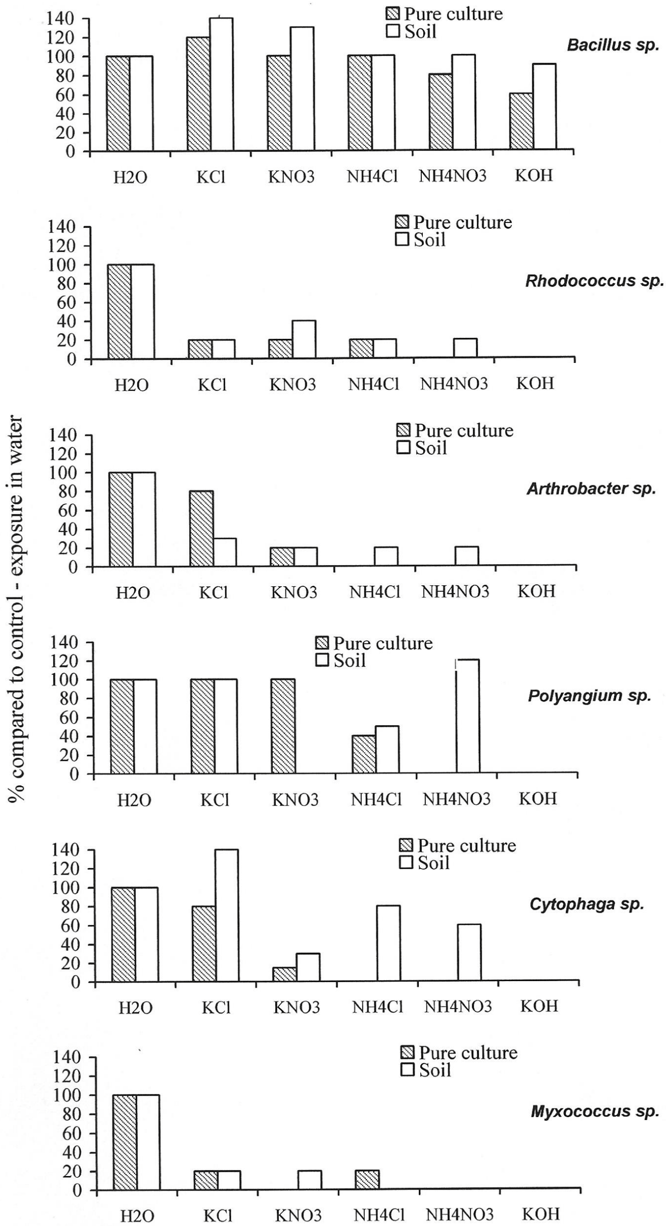

Fig. 4. The influence of short-term action of saturated salt solutions in 1N KOH on the survival of bacterial cultures (in % compared to the control - exposure to H_2O) and bacteria in the soil (in % compared to the control - the number of bacteria in the soil without introducing salt) - (Lapigina and Lisak, 2001).

Conclusion

Based on the above data, it can be concluded that the short-term action of high doses of salt does not have a detrimental effect on saprophytic bacteria in the soil. Nitrogen salts (NH_4NO_3, NH_4NO_3, KNO_3 and NH_4Cl) have the greatest impact on the number and taxonomic composition of saprophytic bacteria, with the greatest effect being the introduction of ammonium nitrate, which is manifested by a 2.5-fold reduction in the number of bacteria and a significant redistribution in the structure of bacteria. Among gram-positive bacteria, bacilli are the most resistant to the action of salt, and among gram-negative bacteria, sliding bacteria. Pure cultures of bacteria are more sensitive to salt than those in the soil.

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HEALTH SAFETY OF BAKERY PRODUCTS

Nela Djonovic[1], Sanja Kocic[2], Svetlana Radevic[2]

Abstract: Nutrition is the main factor in people's health. Food can be both a resource and a great hazard to the health of the entire population.

The Institute for Public Health Kragujevac is an accredited institution for food analysis. In 2023, 472 samples of bakery products were sampled and analyzed; 469 products (99.36%) were microbiologically safe and 460 products (97.45%) were chemically compliant. The increased yeast content was the microbiological reason for the unsoundness of the two bakery products.

Recommended measures: to increase the scope of testing in both microbiological and chemical aspects, by supplementing the current regulations.

Keywords: food, health safety, bakery product

Introduction

Nutrition is considered as the fundamental factor of human health, not only individually but also at the level of the entire population. Additionally, it is a basic human need and an essential prerequisite for maintaining life. From a historical perspective, food has had a particular purpose – serving as a means to satisfy people's hunger with a relatively steady proportion of specific nutritional component shares, i.e., food groups. Alterations in food-taking habits are associated with the development of human society, science, agriculture and industry, and the most dynamic ones were believed to have occured in the 19th century, whereas one of the defining features of the 20th and 21st centuries is related to large migrations of populations of humans as well as demographic, epidemiological and nutritional transition. As regards the total morbidity and mortality rates, there has been a decrease in the participation of communicable diseases on one hand, whereas on the other there has been an increase in the participation of chronic non-communicable diseases (such as cardiovascular diseases, malignant disorders, etc.). A healthy and balanced diet is one of the most significant health resources for all the population categories. It is of particular significance for the youngest age groups at the moment when healthy lifestyles are being formed. It is extremely

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important to maintain adequate energy intake derived from food as well as to provide suitable energy intake for all age groups and both sexes.

Food and nutrition may be a health resource as well, but they may also pose a great health risk to the entire population's health. According to the definition, food consists of food group combinations, whether it is about processed, unprocessed or half-processed foods. Food is everything that enables a human organism to function normally – its life maintenance, growth and development. Each of the given foodstuffs is considered to be food, but not a single foodstuff cannot meet all the body's needs by itself – there is no such thing as a complete foodstuff. As regards its chemical composition, foodstuffs represent combinations of various nutrients, and according to the physiological definition – foodstuffs are mostly regarded as natural products of complex chemical composition, the purpose of which is to provide a normal life to an individual while being in adequate combinations with other food groups. According to the Law on Food Safety, foodstuff is defined as any processed or unprocessed substance used as food or drink complete with water and raw materials used for the production of foodstuffs, spices and additives.

Food of both plant and animal origin can be contaminated by a variety of microorganisms as well as by various chemical agents. Food health safety is determined exclusively using specific laboratory analyses being performed by adequate accredited methods. The examination parameters depend on the kinds of food and groups of foodstuffs and are defined by the national legal regulations – the Law on Food Safety and by a number of several sub-legal acts and Ordinances for each of the food categories separately. Some of the microbiological parameters are *Enterobacteriaceae*, *Salmonella* sp. *Escherichia coli*, a colony count of aerobic bacteria, molds and yeasts such as *Bacillus cereus*, *Listeria monocytogenes*, etc. A variety of chemical contamination can be detected: heavy metals, pesticides, additives, antibiotics, hormones, etc. whereas food may not be harmonized due to other examination parameters as well (degree of acidity, water content, dry matter content, fat content, filling content and likewise).

To control food health safety, food must be properly sampled and transported (in the proper manner) to the laboratory providing accredited microbiological testing and chemical analyses for foodstuffs.

The fundamental aim of this paper is the analysis of laboratory testing and evaluation of bakery products in the year of 2023.

Materials and methods

The research was administered as a retrospective cross-sectional study.

The documentation of the Centre for Hygiene and Human Ecology, Institute of Public Health Kragujevac, was used as the basis for this particular research. The Institute of Public Health Kragujevac, Centre for Hygiene and Human Ecology, is an accredited institution for food testing along with the testing of microbiological and chemical products as well.

The examined microbiological parameters of bakery products are shown in Table 1.

Table 1 – Microbiological parameters of bakery products

Parameter	Unit of measurement	Range	Examination method
Enterobacteriaceae	cfu/g	< 10	SRPS ISO 21528-2:2017
Molds and yeasts	cfu/g	$< 10^2$	SRPS ISO 21527-2:2011

All of the microbiological analyses were performed using adequate examination methods by the currently valid legal regulations (the Ordinance on general and specific food hygiene requirements at any stage of production, processing and transportation, the Official Gazette of the Republic of Serbia, No. 72/2010, 62/2018).

The examined chemical parameters of bakery products are shown in Tables 2, 3 and 4.

Table 2 – Bakery products – yeasts

Parameter	Unit of measurement	Range	Examination method
Degree of acidity	%	-	Ordinance – Article II, paragraph 2
Water content	%	43 max.	Ordinance – Article II, paragraph 1
Dry matter content	%	-	Ordinance – Article II, paragraph 1

Table 3 – Yeast dough and pastry

Parameter	Unit of measurement	Range	Examination method
Water content	%	45 max.	Ordinance – Article II, paragraph 1
Dry matter content	%	-	Ordinance – Article II, paragraph 1
Sodium chloride content	%	-	In the chapter 5.88
Fat content	%	-	Ordinance – Article I, paragraph 15

Tabele 4 – The parameters used in the analysis of burek[1]

Parameter	Unit of measurement	Range	Examination method
Water content	%	-	Ordinance – Article II, paragraph 1
Dry matter content	%	-	Ordinance – Article II, paragraph 1
Sodium chloride content	%	-	In the chapter 5.88
Fat content	%	-	Ordinance – Article I, paragraph 15
Filling content (considering the total product)	%	20 min.	-

[1] *Burek* is a type of baked or fried filled pie made of pastry and stuffed with cottage cheese or ground meat, *the author's comment*.

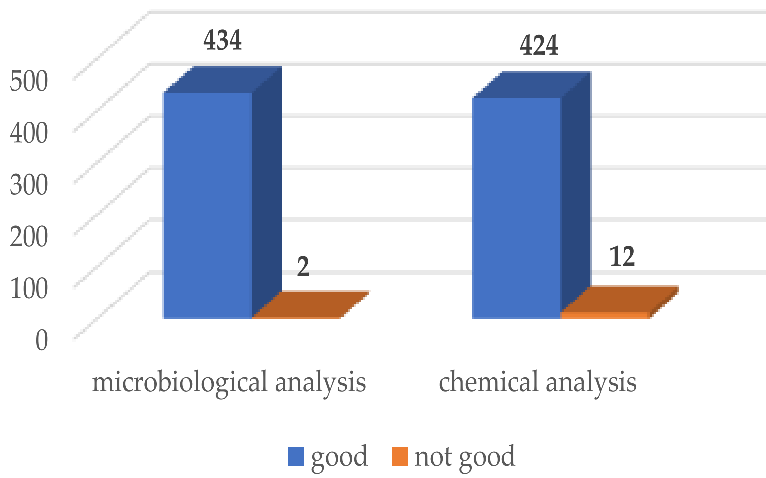
Apart from the above-mentioned parameters for each of the given bakery products, the following organoleptic properties were examined as well:

Appearance, aroma and flavor, the presence of other kinds of mixtures and a declaration (if there is one on the product).

All of the chemical analyses were performed by adequate examination methods by the currently valid legal regulations (the Ordinance on the quality of grain, mill and bakery products and pasta, the Official Gazette of the Republic of Serbia, No. 68/2016, and 56/2018).

Results and discussion

In the year 2023, the sampling and analyses of 436 samples of bakery products were carried out. The results indicated that the microbiological status of the surveyed bakery products was good with 434 (99.5%) samples, whereas the number of the ones produced following the chemical standards was 424 (97.2%). The cause of microbiological spoilage of two bakery products (two loaves of bread) was related to the increased content of yeasts, whereas the decreased filling content in the samples of burek (19.6%) was associated with them not being produced per the chemical standards.



Graph 1. The results of evaluation of bakery products

According to our national legal regulations, bakery products are defined as the ones produced using an adequate technological procedure and made from mill products, and water, with or without bakery yeasts or other ingredients used as leavening agents for raising dough, sodium chloride and other ingredients as well (the Ordinance on the quality of grain, mill and bakery products and pasta, the Official Gazette of the Republic of Serbia, No. 68/2016, 56/2018, Article 2, paragraph 9 – Articles 33-45 refer to the bakery products). Taking into consideration the classification of the bakery products defined in Article 1 of this Ordinance, which is established by their purpose, composition, characteristics and technological procedure, the above-mentioned products are launched into the market as nine groups of products, with bakery products occupying the sixth place. According to this Ordinance (Article 33), bakery products are classified into the following three groups: bread, pastry and other bakery products.

Bakery products are frequently and abundantly used in everyday nutrition of all population categories.

The following reasons may be considered: the availability of bakery products, prices, production methods, storage methods, a fast-paced lifestyle, etc.

These products may contain various kinds of grains with or without gluten, water, salt, sugar, additives, fats, yeasts and various fillings with or without meat and meat products.

Bakery products are not of great epidemiological significance, for they are prepared – baked in high temperatures, which is why the water content in the surface layer is low. Therefore, it does not provide favorable conditions for the growth of microorganisms. If the water content is increased or if the above-mentioned products are not being properly prepared or kept, the growth of yeasts, molds and even Enterobacteriaceae may be expected.

Regular analyses included in the requirements of the Ordinance do not examine the presence of heavy analyses and pesticides (grains are not treated by these means), mycotoxins (that can be found in contaminated grains), the content gluten and content of additives that are added during the production of bakery products. All of these additional products are relevant, so that the physicians could give their complete medical opinion on the health safety of the above-mentioned products.

Conclusion

The examined bakery products demonstrated a considerably high percentage of being by with the norms of national legal regulations and health safety.

The microbiological cause of the lack of health safety of the above-mentioned bakery products was related to an increased content of yeasts in bread, whereas in terms of determining its chemical content – it is associated with burek only and it refers to the decreased amount of filling when taking into consideration the total product which finally affects the overall quality of this particular product.

A proposal of potential measures entails the following: to increase the existing scope of examination in terms of performing further microbiological and chemical analyses, using introducing amendments to the currently valid Ordinances.

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MYCOPOPULATION ON SEED OF WEED PLANTS IN ORGANIC VEGETABLE PRODUCTION CROP

Tanja Vasić[1], Sanja Živković[1], Vera Katanić[2], Bojana Vasilijević[2], Darko Jevremović[2], Jordan Marković[3]

Abstract: What often happens that weeds are not controlled after harvesting the cultivated crop. Thus, they continue to grow unhindered on agricultural land, becoming dangerous hosts for a large number of plant diseases and sources of constant infection. So far, there have been no systematic studies of the mycopopulation of weed seeds in Serbia. In this paper, we present the results of preliminary research. Weed seeds samples were collected during the summer and autumn vegetation of 2023. Isolation was performed using standard phytopathological methods. In these studies of the mycopopulation of weed seeds, a total of 500 seeds originating from ten weed species were examined. Seven genera of fungi were determined in this way: *Penicillium, Fusarium, Alternaria, Cladosporium, Epicoccum, Rhizopus* and *Mucor*.

Keywords: mycoppopulation, weeds seeds

Introduction

Organic agriculture is an agricultural production system whose main goal is to produce products that promote the creation and maintenance of human health. Therefore, it must be based on creating, maintaining and improving the relationship between the environmental impact of the habitat, the impact of the living community on the environment and the mutual impact of living beings, living community in an agroecosystem. Weed control is very important in cooperative relations in organic agriculture, because weeds are the spreaders of various plant diseases and plant pests (Knežević et al., 2008; Ivanović and Ivanović, 2001; Vrbničanin and Božić, 2016). Special attention should be paid to weed control in organic vegetable cultivation, which must be safe for health as a

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necessary and acceptable food for human consumption. Therefore, organic production of vegetables, plant diseases and crop pests must also include proper farming, correct crop rotation, use of controlled plant material, protection of beneficial plants and animals, and creation of favorable conditions for natural enemies and physical and mechanical destruction suppress with weed control (Kačergius, 2003; Knežević et al., 2010; Vrbničanin and Božić, 2021). At the same time, it is very important that weed control is timely and continuous, because otherwise weeds cause serious problems in the crop and its surroundings both during the growing season of the crop and in subsequent growing seasons. A total approach to weed control is only possible if we know under what conditions the presence of weeds in a certain crop causes damage and whether the implementation of weed control measures is economically viable. It also includes a long-term approach to solving the weed problem, because only the implementation of high-quality preventive measures together with weed control can significantly mitigate the damage caused by weeds in a single crop (Hulina, 1993; Kačergius, 2003; Stojanović et al., 2010).

What usually happens here is that weed control is not done before harvest or after harvest of the cultivated crop. Thus, they continue to grow unaided in agricultural land, becoming dangerous hosts for many plant diseases and sources of persistent infection, as pathogens are transferred from them to crops. Ruddy grass growing outside agricultural areas, in neglected gardens and roadsides can also be hosts of plant diseases and sources of infection of crops.

The purpose of our work is to study the micropopulation of weeds in the organic production of tomatoes and peppers in an open field, because weeds occur in the harvest of peppers and tomatoes and are spreaders of pathogenic fungi on all surrounding crops, especially vegetables harvests.

Materials and methods

Samples of weed seeds were collected in the village of Trmčare (near Kruševac), from the plot of the Živković family during the summer growing season and from the land in the autumn growing season of 2023, where peppers and tomatoes were grown according to the principles of organic production. Surrounding the vegetable garden were cornfields. Wash the weed seeds thoroughly under running water. After washing, weed seeds are ready for isolation. Prepared seed samples were disinfected with 96% ethanol for 10 seconds and 1% sodium hypochlorite (NaOCl) for 1 minute, and then washed three times in sterile distilled water. They were then dried on sterile filter paper

and placed on potato dextrose agar (PDA) medium with streptomycin. Ten seeds per weed species were placed in Petri dishes in five replicates. They were kept in a thermostat at 25°C. Observations were made every 3 days, and most mycelial samples were developed for up to 14 days. The developed mycelium was examined on a new PDA substrate, and after initial growth, the mycelial tip was reseeded on the PDA. Microscopic examination was performed using Olympus CX31 microscopes. Morphological identification of fungi to genus was done using a standard key.
Calculated isolation frequency in percentage using the formula Vrandečić et al. (2011):

$$\text{(\%) Isolation frequency} = \frac{\text{Number of segments containing the fungal species}}{\text{Total number of segments used in the isolation}} \times 100$$

Results and discussion

A total of 500 seeds from ten weed species were examined in these weed seed micropopulation studies. These are the most common weeds in tomato and pepper plants: *Hibiscus trionum* L., *Xanthium strumarium* L., *Portulaca oleracea* L., *Eleusine indica* L., *Artemisia vulgaris* L., *Datura stramonium* L., *Clematis vitalba* L., *Raphanus raphanistrum* L ., A high percentage of fungal colonies formed around the weed seeds of *Salvia verticillata* L. and *Saponaria officinalis* L. (Table 1). In that context, seven different genera of fungi were identified: *Penicillium, Fusarium, Alternaria, Cladosporium, Epicoccum, Rhizopus* and *Mucor*. Among them, the genera *Fusarium, Alternaria, Cladosporium* and *Epicoccum* can cause great damage to plants grown in the field, and the genera *Penicillium, Rhizopus* and *Mucor* can cause serious damage to stored products.

Table 1. Frequency of fungal isolation on weed seeds

Weed species	**Number of samples Plant part - seeds**	**Fungi species**	**(%) Isolation frequency**
***Hibiscus trionum* L.**	50	*Penicillium* sp.	**26**
		Alternaria sp.	**24**
***Xanthium strumarium* L.**	50	*Alternaria* sp.	**8**
		Penicillium sp.	**10**
		Cladosporium sp.	**22**
		Rhizopus sp.	**6**

***Portulaca oleracea* L.**	50	*Penicillium* sp.	**24**
		Cladosporium sp.	**10**
***Eleusine indica* L.**	50	*Alternaria* sp.	**10**
		Penicillium sp.	**20**
		Cladosporium sp.	**16**
Artemisia vulgaris L.	50	*Alternaria* sp.	**20**
		Cladosporium sp.	**10**
		Fusarium sp.	**6**
		Epicoccum sp.	**16**
***Datura stramonium* L.**	50	*Mucor* sp.	**14**
		Alternaria sp.	**14**
		Fusarium sp.	**22**
***Clematis vitalba* L.**	50	*Alternaria* sp.	**10**
		Penicillium sp.	**24**
		Cladosporium sp.	**20**
***Raphanus raphanistrum* L.**	50	*Alternaria* sp.	**24**
		Cladosporium sp.	**10**
		Epicoccum sp.	**18**
***Salvia verticillata* L.**	50	*Alternaria* sp.	**24**
		Penicillium sp.	**14**
		Cladosporium sp.	**20**
		Mucor sp.	**8**
***Saponaria officinalis* L.**	50	*Alternaria* sp.	**26**
		Penicillium sp.	**8**
		Cladosporium sp.	**22**
		Fusarium sp.	**38**

It usually happens here that the weeds are not controlled after the harvest or harvest of the cultivated crop. Thus, they continue to grow unaided in agricultural land, becoming dangerous hosts for many plant diseases and sources of persistent infection, as pathogens are transferred from them to crops. Ruddy grass growing outside agricultural areas, in neglected gardens and roadsides can also be hosts of plant diseases and sources of infection of crops (Triole et al., 2022). Mirzaee et al., 2009 reported that *Albugo Candida* causes white rust of *Erysimum crassicaule* in Iran.

It is known that some pathogenic fungi, such as those causing cabbage (*Plasmodiophora brassicae*), develop in the roots of other single cruciferous plants, such as cowpea (*Capsella Bursa pastoris*), wild radish (*Raphanus raphanistrum*), wild mustard (*Sinapis arvensis*) and others, so they must be removed from the cabbage. As soon as the disease appears and for the next 6-7 years, if you want to grow cruciferous plants in this place, you must destroy all the cruciferous plants so that the fungus is also destroyed (Hulina, 1993; Ivanović and Ivanović, 2001; Stojanović et al., 2010; Knežević et al., 2010).

The fungus *Erysiphe polygoni* parasitizes hundreds of plant species (375 species from 175 genera) and causes diseases in Fabaceae (especially attacking peas) and Cucurbitaceae. The fungus *Sclerotinia trifoliarum* parasitizes many weeds, for example: *Ranunculus repens, Holosteum umbellatum, Capsella Bursa pastoris, Veronica persica, Plantago lanceolata, Senecio vulgaris, Sonchus* sp. and *Taraxacum officinale* (Stojanović et al., 2010; Knežević et al., 2010). Through the weeds, the fungus can spread, causing a very dangerous disease, clover cancer. This disease causes severe damage to crops of different types of clover, alfalfa, asparagus and beans. Its stem is thick compared to the average height, the leaves are smaller and covered with spermagonia and ecido. On milkweed, the parasite lives in the underground part of the stem in the form of a mycelium, which can produce ecidium on the leaves of newly formed shoots every spring. Echidiospores infect peas and basidiospores infect milkweed (Stojanović et al., 2010). The fungus *Alternaria brassicae* (Berk) Sace attacks cabbage, cauliflower, canola, as well as other cruciferous vegetables and even weeds, where they overwinter as conidia and thus the infection is renewed the following year (Hulina, 1993).

Conclusion

This paper presents preliminary results on the micropopulation of weed seeds from organic production of tomato and pepper crops. Fungi have been isolated from the seven genera *Penicillium, Fusarium, Alternaria, Cladosporium, Epicoccum, Rhizopus* and *Mucor*, which can infect crops in the field and in storage, where they can cause serious damage.

Therefore, the destruction of weeds that are potential hosts of plant diseases is a very important agrotechnical measure. This must also be done after removing the crop and after harvesting. Mainly to reduce the infectious inoculum of these parasites and prevent their spread from weeds to crops. For diseases that have multiple hosts during development, weeds are particularly important for their persistence and spread of infection. Crop diseases usually overwinter on weeds associated with crops, where they cause damage. Thus, most of the host weeds of plant pathogenic fungi are found in the same plant families as the cultivated species.

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CONTRIBUTION TO THE RESEARCH OF THE FLORA AND HABITAT CHARACTERISTICS OF SP "BORAČKI KRŠ" BORAČ

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Abstract: The Natural Monument "Borački krš", encompasses a part of the Rudnik-Kotlenik region and it is located in the southwestern Šumadija between Rudnički, Kotlenik and Gledić mountains on the municipality of Knić. In the research area, 117 taxa were identified, classified into 41 families. The most numerous species belong to the families Asteraceae (16) and Lamiaceae (9). The forest is hemicrypto-terrophytic with a strong participation of fanerophytes. The high percentage of terophytes and fanerophytes is particularly characteristic, as it indicates a very warm and dry vegetation period of habitat. Invasive species are *Erigeron annuus* (L.) Pers., *Amaranthus retroflexus* L. and *Robinia pseudoacacia* L.

Keywords: Borački krš, plant species, life forms, invasive species

Introduction

The Natural Monument "Borački krš" covers a part of the Rudnik-Kotlenik area, and it is located in the southwestern Šumadija between Rudnik, Kotlenik and Gledić mountains on the territory of the municipality of Knić and includes the municipality of Borač. The geographical location of monument of nature "Borački krš" is shown in Figure 1.

Figure 1. Geographical location of monument of nature "Borački krš"

Borački krš represents an igneous formation formed by bloating dense lava masses whose parts have eroded over time, which gave this elevation its

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recognizable appearance. Borački krš - represents a unique geological and geomorphological phenomenon of the remains of tertiary volcanic relief in Šumadija and specific remains of the edge of the quartzlatite caldera with traces of columnar secretion in rocks and numerous remains of bizarre shapes. Since 1995 it has been kept in the Inventory of Geoheritage Objects of Serbia in the group geomorphological objects of erosive character (Karamata, Mijović, 2005).

According to Brković, (2015), 59 taxa were identified in the investigated area. Among the taxonomies are: *Allium carinatum* L., *Ruscus aculeatus* L. and *Trifolium arvense* L. on the List of plant species and subspecies in the flora of northwestern Serbia and Šumadija, which were included in the European Red List (Bilz et al 2011). While the *Carduus candicans* are Waldst. & Kit., *Crataegus monogyna* Jacq. and *Scabiosa fumarioides* Vis. & Pančić on the List of Strictly Protected Species and Subspecies (Službeni glasnik RS. br. 5/10).

Materials and methods

Going out on the field and sampling of the material was carried out three times in the summer, late summer and autumn aspects.

The determination of plants was made according to the keys EURO + MED. (2006), Josifović (1970-1977), Javorka & Csapody (1991) and Domac (2002).

The affiliation of the taxa to the appropriate life form was determined according to the System Raunkiaer (1934), which was supplemented by Mueller-Dombois and Ellenberg (1974), and for taxa at the Level of Serbia by Stevanovic (1992A).

Results and discussion

After collecting data from earlier research and after determining newly collected plant material in the wider area of SP "Borački krš" 117 taxa were identified. These are: *Sambucus ebulus* L., *Sambucus nigra* L., *Amaranthus retroflexus* L., *Allium carinatum* L., *Eryngium campestre* L., *Carum carvi* L., *Orlaya grandiflora* (L.) Hoffm., *Torilis japonica* (Houtt) DC, *Conium maculatum* L., *Cynanchum vincetoxicum* (L.) Pers., *Arum maculatum* L., *Hedera helix* L., *Asarum europaeum* L., *Ruscus aculeatus* L., *Polygonatum odoratum* (Mill.) Druce, *Asplenium adiantum-nigrum* L., *Asplenium trichomanes* L., *Carduus candicans* W. et K., *Centaurea stoebe* subsp. *australis* (A. Kerner) Greuter, *Hypochoeris radicata* L. subsp. *radicata*, *Sonchusa arvensis* L., *Achillea milefolium* L., *Arctium lappa* L., *Cirsium arvense* L. (Scop.), *Lactuca serriola* L., *Artemisia vulgaris* L., *Echinops sphaerocephalus* L., *Taraxacum campylodes* G.E.Haglund, *Cichorium intybus* L., *Carthamus creticus* L., *Filago arvensis* L., *Erigeron annuus* (L.) Pers., *Artemisia absinthium* L., *Carpinus betulus* L., *Carpinus orientalis* Mill., *Echyum vulgare* L., *Lunaria rediviva* L., *Capsella bursa-pastoris* (L.) Medik., *Alyssum petraeum*

Ard, *Campanula trachelium* L., *Campanula rapunculoides* L., *Scabiosa fumarioides* Visiani et Pančić, *Dianthus armeria* L., *Lychnis coronaria* (L.) Desr., *Petrorhagia saxifraga* (L.) Link., *Silene latifolia* Poir, *Stellaria media* (L.) Vill., *Scleranthus perennis* L., *Convolvulus arvensis* L., *Cornus mas* L., *Cornus sanguinea* L., *Hylotelephium telephium* (L.) Ohba, *Sedum hispanicum* L., *Sedum acre* L., *Sempervivum marmoreum* Griseb., *Dioscorea communis* (L.) Caddick & W., *Euphorbia cyparissias* L., *Lathyrus tuberosus* L., *Trifolium pratense* L., *Securigera varia* (L.) Lassen, *Robinia pseudoacacia* L., *Vicia grandiflora* Scop., *Trifolium arvense* L., *Trifolium patens* Schreb., *Lembotropis nigricans* (L.) Griseb, *Quercus frainetto* Ten., *Quercus cerris* L., *Geranium columbinum* L., *Geranium lucidum* L., *Hypericum perforatum* L., *Stachys germanica* L., *Stachys recta* L., *Ballota nigra* L. subsp. *nigra*, *Stachys officinalis* (L.) Trevis. ex Briq., *Prunella vulgaris* L.subsp. *vulgaris*, *Salvia verticillata* L., *Clinopodium vulgare* L., *Sideritis montana* L., *Acinos alpinus* (L.) Moench, *Malva sylvestris* L., *Fraxinus ornus* L., *Fraxinus excelsior* L., *Pinus nigra* Arn., *Digitalis ferruginea* L., *Linaria genistifolia* (L.) Mill. ssp. *sofiana*, *Plantago media* L., *Plantago lanceolata* L., *Plantago major* L., *Agrostis capillaris* L., *Chrysopogon gryllus* (L.) Trin., *Danthonia provincialis* Lam. et DC, *Festuca vallesiacea* Schleich., *Melica ciliata* L., *Poa nemoralis* L., *Melica uniflora* Retz, *Cynosurus echinatus* L., *Rumex acetosella* L., *Rumex crispus* L., *Polypodium vulgare* L., *Primula acaulis* (L.) Hill, *Helleborus odorus* Waldst. & Kit., *Clematis vitalba* L., *Consolida regalis* Gray, *Fragaria vesca* L., *Potentilla argentea* L., *Cotoneaster integerrimus* Medicus, *Crataegus monogyna* Jacq., *Rubus ulmifolius* Schott, *Rosa canina* L., *Prunus avium* L., *Galium corrudifolium* Vill., *Acer tataricum* L., *Acer campestre* L., *Verbascum lychnitis* L., *Viola arvensis* Murr.

Determined plants are classified into 41 families. The expected most abundant plant family is Asteraceae (39%), followed by Lamiaceae (22%), Fabaceae and Poaceae (20%), Apiaceae, Caryophyllaceae and Rosaceae (15%), Plantaginaceae (12%) and Crassulaceae (10%). Other families are represented by less than 10% of the total. The overview and percentage of plant families on the monument of nature "Borački krš" is shown in Table 1.

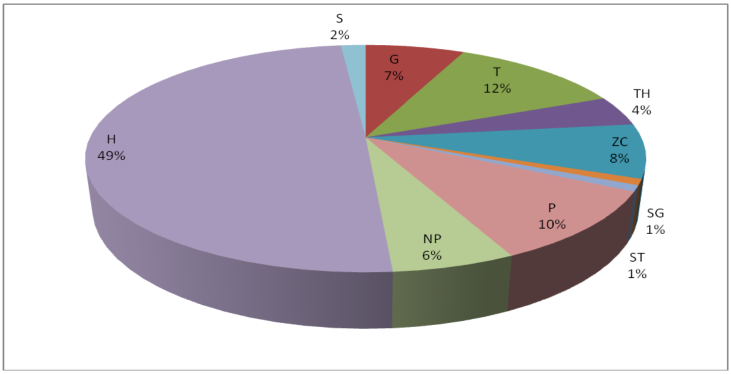
Table 1. Percentage of representation of plant families in the area of monument of nature "Borački krš"

Asteraceae	**16**	39%
Adoxaceae	**2**	5%
Amaranthaceaea	**1**	2%
Amarylidaceae (Liliaceae)	**1**	2%
Apiaceae	**6**	15%
Apocyniaceae	**1**	2%
Araceae	**1**	2%
Araliaceae	**1**	2%

Aristolochiaceae	**1**	2%
Asparagaceae	**2**	5%
Aspleniaceae	**2**	5%
Betulaceae	**2**	5%
Boraginaceae	**1**	2%
Brassicaceae	**3**	7%
Campanulaceae	**2**	5%
Caprifoliaceae	**1**	2%
Caryophyllaceae	**6**	15%
Convolvulaceae	**1**	2%
Cornaceae	**2**	5%
Crassulaceae	**4**	10%
Dioscoreaceae	**1**	2%
Euphorbiaceae	**1**	2%
Fabaceae	**8**	20%
Fagaceae	**2**	5%
Geraniaceae	**2**	5%
Hypericaceae	**1**	2%
Lamiaceae (Labiatae)	**9**	22%
Malvaceae	**1**	2%
Oleaceae	**2**	5%
Pinaceae	**1**	2%
Plantaginaceae	**5**	12%
Poaceae	**8**	20%
Polygonaceae	**2**	5%
Polypodiaceae	**1**	2%
Primulaceae	**1**	2%
Ranunculaceae	**3**	7%
Rosaceae	**6**	15%
Rubiaceae	**1**	2%
Sapindaceae (Aceraceae)	**3**	7%
Scrophulariaceae	**1**	2%
Violaceae	**1**	2%

The results of this study showed that the biological spectrum of the hornbeam forest with the presence of cera malt has the following relationships: hemicryptophytes 49%, fanerophytes and nanofanerophytes 16%. (terophytes) and (terophytes/hamefites) 16%, herbaceous hamephytes 8%, geophytes 7%.

The forest is hemicrypto-terrophytic with a strong participation of fanerophytes. The high percentage of terophytes and fanerophytes is particularly characteristic, as it indicates a very warm and dry vegetation period of habitat. The percentage of the representation of life forms of plants in the area of monument of nature "Borački krš" is shown on Graph 1.



Graph 1. Percentage of the representation of life forms of plants in the area of monument of nature "Borački krš" P (fanerophytes), NP (nanofanerophytes), ZC (herbaceous hamephytes), H (hemicryptophytes), G (geophytes), T (therophytes), TH (terophytes/hamefites) S scandenophytes (climbers and lians)

Protected plant species in the "Borački krš" are: *Acer hyrcanum* subsp. *intermedium*, *Asarum europaeum* L., *Carduus candicans* W. et K., *Crataegus monogyna* Jacq, *Hypericum perforatum* L., *Primula acaulis* (L.) Hill, *Ruscus aculeatus* L. and *Scabiosa fumarioides* Visiani et Pančić.

Protected wild plant species related to wild flora traffic control (Vuković, 2012) are: *Achillea millefolium* L., *Cornus mas* L., *Sedum acre* L., *Trifolium pratense* L., *Hypericum perforatum* L., *Ruscus aculeatus* L., *Malva sylvestris* L., *Plantago lanceolata* L., *Plantago major* L., *Plantago media* L., *Helleborus odorus* Waldst. &Kit. *Crataegus monogyna* Jacq., *Fragaria vesca* L., *Rosa canina* L., *Sambucus nigra* L. and *Digitalis ferruginea* L.

According to the list of invasive species of Serbia (Stojanović et al., 2021), three species are present in the area of "Borački krš": *Erigeron annuus* (L.) Pers., *Amarantus retlofexus* L. and *Robinia pseudoacacia* L.

Conclusion

In the area of the natural monument "Borački krš" based on literature reviews and field research, 117 plant taxa were noted within 41 plant families.

The expected most abundant plant family is Asteraceae (39%), followed by Lamiaceae (22%), Fabaceae and Poaceae (20%), Apiaceae, Caryophyllaceae and Rosaceae (15%), Plantaginaceae (12%) and Crassulaceae (10%). Other families are represented by less than 10% of the total. The results of this study showed that the biological spectrum of the hornbeam forest with the presence of cera malt has the following relationships: hemicryptophytes 49%, fanerophytes and nanofanerophytes 16%. (terophytes) and (terophytes/hamefites) 16%, herbaceous hamephytes 8%, geophytes 7%.

In the study area, 16 taxa protected wild plant species were identified, as well as 3 types of invasive plants.

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EMPOWERING SMALL-SCALE DAIRY PROCESSORS: A COMPREHENSIVE ANALYSIS OF TRAINING IMPACT AND PARTICIPANT FEEDBACK

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Abstract: To overcome obstacles faced by small scale dairy processors (SSDPs) in Serbia, a training program was implemented. Two phases of training evaluation (reaction and learning) were conducted. A demographic breakdown showed that it was difficult to achieve gender and educational projections. Reaction evaluation demonstrated the highest satisfaction with the overall quality of the training. The main concern was related to the training duration, which led to plans for e-learning training. It was recognised that it is difficult to attract young people, women and participants with low level of education.

Keywords: Small-scale dairy processors, dairy education, training evaluation, lifelong learning

Introduction

The COVID-19 pandemic has disproportionately affected small scale dairy processors (SSDPs) in Serbia, presenting them with unique challenges in adapting to changing market dynamics, particularly in online sales. Recognizing the hurdles faced by these producers, a region-specific approach was adopted to address their needs (Miloradovic et al., 2024). Tailored training programs were developed and delivered to SSDPs. However, the importance of training goes beyond its delivery - it also extends to the crucial step of measuring evaluating and reviewing its impact and effectiveness.

Kirkpatrick’s evaluation model, with its four distinct phases – reaction, learning, behaviour, and outcomes – serves as a comprehensive framework for evaluating the effectiveness of training programs (Kirkpatrick & Kirkpatrick, 2006). The use of the first two phases of Kirkpatrick’s model remains widespread as they are practical and provide valuable insights into the immediate reactions and learning outcomes of training programs.

The objective of this study was to evaluate the impact of face-to-face training on the small-scale dairy processors in terms of the response (level 1) and learning (level 2) phases of Kirkpatrick’s training evaluation model. The aim was to explore whether the existing content and format of the training was appropriate for the specific target group, to examine the contribution of the training to the objectives of the COV-183 PEER project, to assess the potential for extending the training program beyond the duration of the project, and gather insights into specific areas for improving future training initiatives.

Materials and methods

Training content

The training for SSDPs was designed based on the results of a thorough training needs assessment (Miloradovic et al., 2024). The main areas covered in the training included hygiene, cleaning and sanitation, infrastructure and prerequisite programs, dairy products labelling, procedures required for registering a small-scale dairy processing unit. Emphases was also placed on the importance and the ways in which high quality raw milk is preserved, and how it affects the quality of cheese. Recognizing the increasing significance of the digital landscape, the training equipped participants with an understanding of quality considerations for online customers. Various options for online sales were explored to help small-scale dairy producers adapt to evolving market trends.

Design, recruitment and delivery of the training

To optimize the effectiveness of the training, a group size of 10 to 15 participants was maintained. As outlined in the project proposal, of the aim was to have at least 70% female participants, with at least 50% having a lower than secondary education. Additionally, all participants were required to be at least 18 years old and owners or members of an agricultural household in Serbia.

Participants for the training were recruited through diverse methods. The recruitment information was shared through a dedicated social media channel: "Mlečna zajednica". Personal contacts with small-scale dairy producers were provided by the local Chambers of Commerce from Zaječar, Niš and Valjevo. Additionally, the participants were recruited at the Balkan Cheese Festival, and some participants were already involved in the training needs assessment.

In 2023, trainings were conducted at four distinct locations: Belgrade (July), Zaječar (October), Niš (October), and Valjevo (November). Each training lasted from 9AM to 4PM, including a half- hour coffee break and a one-hour lunch break. In total, 43 SSDPs completed the training.

Level 1 evaluation – Reaction

After the training, the evaluators distributed anonymous questionnaires with three single-choice questions on gender, age, and education to all participants. Additionally, participants responded to eight questions using a five-point Likert scale to rate their satisfaction with various aspects of the training quality: content, organization, duration, clarity, applicability, material, communication, and overall impression. The questionnaire also included a section for written comments, which allowed an assessment of subjective individual responses to the training program.

Figure 1(A, B and C) displays the total number and demographic breakdown of participants, encompassing gender, education and age, at the different locations. Figure 1(F) presents the calculated average scores reflecting participants' satisfaction with the quality of the training programme.

Level 2 evaluation - Learning

The test questions were developed in line with the objectives of the training program and encompassing all the key educational elements presented during the training. The test consisted of ten questions, each of which offered three or four answers with a single correct answer. The training participants were instructed to complete a pre-test before the training and a post-test immediately afterwards, to determine their level of understanding. The content of the two tests was identical. They were designed in different colours for differentiation. Although they were anonymous, both tests were numbered so that the evaluators could allocate them to each other for the purpose of assessing individual knowledge increase. The individual knowledge increase was calculated as follows:

$$\text{Knowledge increase}(\%) = \frac{\text{N correct answers, post\_test} - \text{N correct answer, pre\_test}}{\text{N correct answers, pre\_test}} * 100\%$$

The average test scores for each location were derived by calculating the average of the individual participant scores, both for the pre- and post-tests. Test scores, as well as the knowledge increase, were presented in Figure 1 (D, E) for each city in which the training session was conducted.

Results and discussion

Demographic structure of participants

It turned out to be a major challenge to attract female dairy producers to participate in training, and although the projection was at least 70%, only 58% were women and 42% men. It is believed that the challenge is that in Serbia, particularly in rural areas, gender inequalities limit women to their traditional roles as housewives and mothers while neglecting their educational individuality (Cvetkovic, 2015). Therefore, efforts should be made in the future to overcome these barriers.

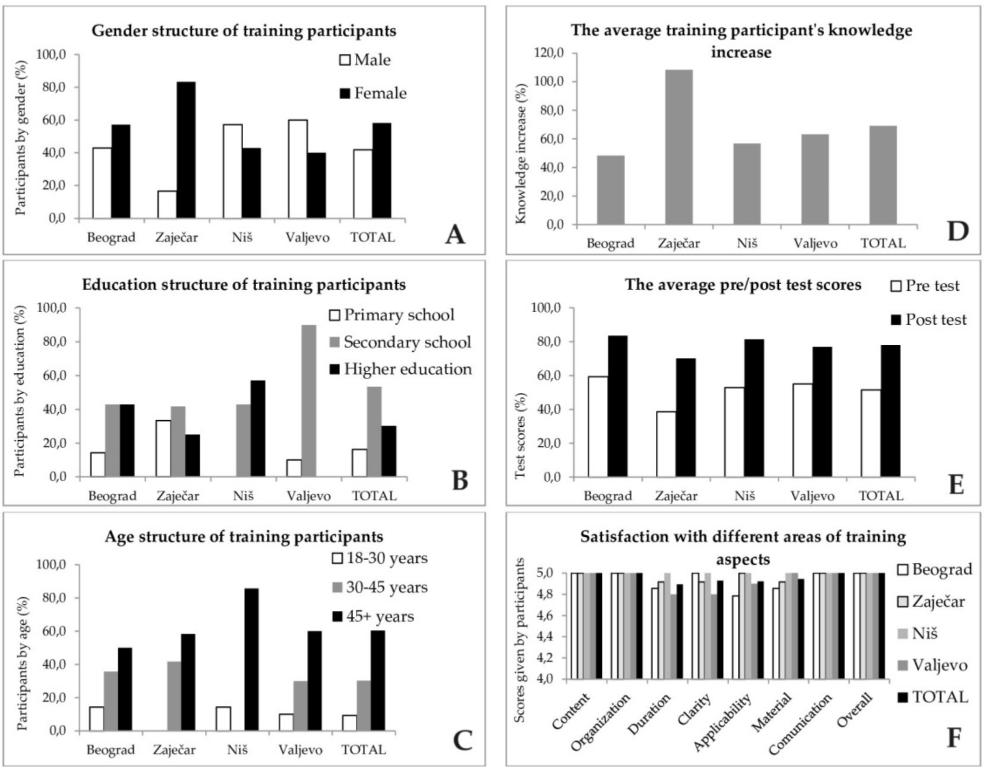

Figure 1. Demographic structure of training participants regarding the gender (A), education (B) and age (C); Knowledge increase (D), pre/post test scores (E); Participant's satisfaction with different areas of training aspects (F);

The following age groups (years) were: 18-30 (9%), 30 to 44 (30%) or ≥ 45 (61%). The senior category (≥ 45 years) was the most numerous age group, and it is very positive that this group is still motivated to upskill or retrain. However, it would be of great importance to attract more young people, since empowering this category with knowledge would contribute to the sustainability of small scale dairy processing and rural development in general. The level of education also varied: 6% had completed primary school, 54% had completed secondary school, and 30% had a university degree.

Reaction

As outlined by Kirkpatrick and Kirkpatrick (2006), reaction evaluation at this level is essential for enhancing the training performance in the future. When asked about the overall quality of the training, 100% of participants gave the maximum score – 5 points. In addition, the most frequent written comment in the questionnaire was: „There should be more training of this type in the future". This indicates that the participants gained confidence, felt comfortable, and would like to come back to learn more. This fact is found to be extremely important, because small scale dairy producers come from a very different backgrounds and need to be provided with training that is relevant and low-stress (Dutta, 2018). However, 19% of participants reported a decrease in the maximum score on certain aspects of training quality, as illustrated in Figure 1(F). This decrease was primarily attributed to the length of the training (12%). Participants found it difficult to commit the entire day to the training, especially considering the impact of losing a day of their farm activities. To address this challenge, an e-learning training course is currently being developed and will be launched in April 2024.

Learning

The results of post-test indicated an average score of 78%, with particularly notable performance at two sites, Belgrade and Niš, where the scores exceeded 80% (Figure 1(E)). This can be considered very satisfactory from a training success perspective. However, it is important to note that these two locations were distinguished by an above average proportion of highly educated trainees (57%). The high learning outcomes can be attributed to their high initial knowledge, but also to their advanced learning skills and educational background.

While in Zaječar training participants scored lower on both the pre- and post-test, their average knowledge increase was nearly double the overall average (Figure 1E)). This suggests that this group had the greatest knowledge benefit from the training. Aligning with the planned target demographic (low educated women), this emphasizes the need to increase efforts to attract and support for this specific group in the future. Based on the present study, but also as stated in the published literature, it appears that the number of educational opportunities in general needs to be increased, specifically through online and practical training (Dutta et al., 2021).

Conclusion

In general, the training participants were highly satisfied with the training and expressed a desire for trainings of this kind in the future. The main remark – the long duration should be remedied by introducing an e-learning training programme. It would be of great importance to attract more young people to participate in the trainings, for contributing the sustainability of small-scale dairy processing. In addition to young people, the aim of the future efforts will be to empower women to overcome the constraints of social norms and promote their personal development in this specific area of lifelong learning.

Acknowledgement

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MICROBIOLOGICAL SAFETY OF FOOD OF ANIMAL ORIGIN IN BELGRADE RESTAURANTS AND CATERING FACILITIES

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Abstract: Total of 40 samples of food of animal origin were analyzed during monitoring in 2023. Out of 40 samples in 3 (7.5%) ice cream samples was dectected presence od Enterobacteriacae. All 30 samples of sandwiches and sushi examined for the presence of *Listeria monocytogenes* and *Salmonellae* spp. were negative. It can be concluded that food of animal origin in Belgrade restaurants was safe for consumption, except 3 (7.5%) samples of ice cream who were not satisfactory to hygiene criteria.

Keywords: food of animal origin, sandwiches, sushi, ice cream, safety

Introduction

Food of animal origin plays an important role in the human diet. Nutritionally, it is important source of high-quality proteins and excellent sources of vitamins and minerals. In addition, food of animal origin is in general more distinctive in flavor and texture. According to a recent Food and Agriculture Organization (FAO) report, foods of animal origin are needed to ensure human nutrition and health (FAO, 2023). In fact, they provide proteins of high biological value, precious fatty acids and a rich supply of vitamins and minerals. This FAO report 'Contribution of terrestrial animal source food to healthy diets for improved nutrition and health (2023) regarding the usefulness of food of animal origin in nutrition - is the first of the four documents requested in October 2020 by the FAO Committee on Agriculture.

Important fact is that more than 900 million tons of food ends up in the trash every year, according to a new United Nations (UN) report. The Food Waste Index of the UN Environment Programme revealed that as much as 17 percent of the food that reaches consumers through stores and restaurants ultimately ends up in the trash. Additionally, approximately 60 percent of food is wasted at home (BBC, 2021). At the same time, animal husbandry is faceing

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the challenges in a One Health approach which can not neglect the close relationship between human health, animal health and well-being, and environmental protection.

Nowadays, we are challenging the changes in human diet, as well. Dietary habits reflect individual food preferences and are often related to culture, education, socioeconomic background, and health status (Krause et al., 2015). Dietary habits may also be influenced according to life stage and lifestyle factors including physical exercise and social engagement. They have been changing in recent years due to covid pandemia, lifestyle, market offer, economic status. Many people decide to eat prepared dishes at restarants due to lack of time to cook at homes. Tourists often visit and consume food in restarurants (Krause et al, 2015).

In the Republic of Serbia, according to the data of the Agency for Business Registers, there are a total of 8.214 catering establishments in Belgrade, including 5.689 restaurants and cafes. Additionally, there are 459 hotels and 2.066 cafes operating in the capital of Serbia. This is a huge number of restaurants which are visited by citizens and tourists. The Washington Post pointed out that Michelin Guide presented its first guide to restaurants in Belgrade, and although Belgrade was not awarded by any stars, 14 restaurants in the capital were marked as "recommended" and three more were added to that list at the beginning of December last year. The year 2022 was a record year for the number of tourists in Belgrade, but also for earnings. Already in October, Belgrade surpassed the year 2019, which in terms of world tourism was considered the best. According to Tourist Belgrade Organisation in October, the number of tourists who visited Belgrade exceeded one million. This is 75 percent more arrivals and 50 percent more overnight stays than the previous year, in 2021.

Concedering the large number of resaturants, tourists and citizens, as well habits of consuming prepared dishes in catering facilities, the aim of this article was to examine the microbiological safety of food of animal origin in Belgrade restaurants.

Materials and methods

Material represented 40 samples of food of animal origin from reastaurants and catering facilities in Belgrade. Out of 40 food sampes, 15 were sadwiches, 15 were sushi and 10 samples of ice cream. Samples were colected during monitoring in 2023. Samples were trasported in refrigirated conditions under 4 °C. The analyses were started after delivery in the laboratory. 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). ISO standard methods were used to determine presence microorganisms in food samples. The SRPS EN ISO 11290-2 method was used to detect *Listeria monocytogenes;* SRPS EN ISO 6579 method was used to detect *Salmonella* spp. 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 cream production process.

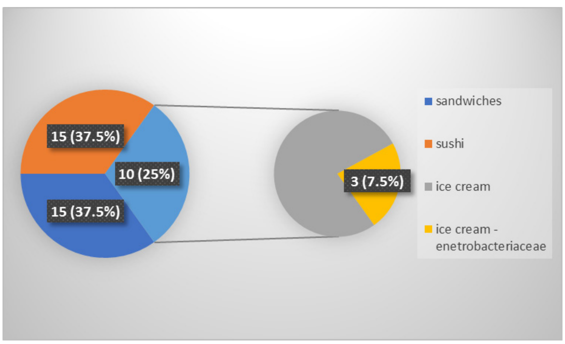
Results and discussion

The obtained results are sumarized in Table 1 and Graph 1 for each microbiological parameter and thype of food sample.

Table 1. Results of microbiological analysis of food of animal origin

Food	Microorganisms											
	L. monocytogenes in 25 g				*Salmonellae* spp. in 25 g				Enterobacteriacae >100 log CFU/g			
	positive		negative		positive		negative		positive		negative	
	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
Sandwiches	0	0	15	37.5	-	-	-	-	-	-	-	-
Sushi	0	0	15	37.5	-	-	-	-	-	-	-	-
Ice cream	-	-	-	-	0	0	10	25	3	7.5	7	
Total	0	0	30	75					3	7.5	7	17.5

(-)-not analyzed for the presence of this microorganism



Graph 1. Results of microbiological analysis of food of animal origin

A sandwich is a widely popular food, usually consisting of vegetables, sliced cheese or meat, placed on/or between slices of bread, or more generally any dish wherein bread serves as a container or wrapper for another food type (Abelson, 2006). The sandwich began as a portable, convenient "finger food" in the Western world, but its popularity has spread globally over time. Sandwiches are a popular type of lunch food, taken to work, school, or picnics to be eaten as part of a packed lunch. The bread may be plain or be coated with condiments, such as mayonnaise or mustard, containing cheese, ham or boiled eggs to enhance its flavour and texture. Sandwiches are considered potentially hazardous foods because pathogenic bacteria (e.g., *Salmonella* spp. and *Listeria monocytogenes*) may be present in some common ingredients such as eggs, salad dressing, sliced deli meats, fresh vegetables, etc (CFS, 2022). As well as being homemade, sandwiches are also widely sold in various retail outlets and can be served hot or cold.

Sushi (Japanese: 寿司, su-vinegar and Me/shi-rice) is a traditional Asian food, which originates from Southeast Asia, and has been used in Japan for over 2,000 years. It is prepared from rice seasoned with vinegar, salt, sugar, and seafood, which is usually raw, sometimes thermally processed. It belongs to Ready-to-eat (RTE) foods a group of food products that are pre-cleaned, precooked, mostly packaged and ready for consumption without prior preparation or cooking.

According to the 2022 US Food Code (FDA, 2022), RTE foods should be in an edible form without an additional preparation step to achieve food safety. Foods in this category usually contain raw materials of animal origin, such as eggs, fish, meat, poultry and ratites, and must be cooked to allow the lowest internal temperature to reach a minimum temperature, for a minimum holding time, during manufacturing to destroy microorganisms of public health concern. In an industrial setting, the cooking step is achieved by thermal processing using steam, hot water, microwave, or infrared. The thermal process should be designed by a thermal process authority and should ensure a minimum lethality (F_0) for the target microorganism (usually a foodborne pathogen). A properly processed and packaged RTE food should be free of the foodborne pathogen and ready for consumption (Huang and Hwang, 2012).

Among common foodborne pathogens, such as *Listeria monocytogenes*, pathogenic *Escherichia coli* and *Salmonella* spp., *L. monocytogenes* is the major concern for refrigerated RTE foods (Huang and Hwang, 2012). From a risk assessment perspective, the health sensitivities of specific consumer categories, such as young, old, pregnant and immunocompromised (so-called YOPI), deserve particular attention since even low contaminating loads of pathogens could have fatal outcomes (Lund, 2015).

Our results showed that *L. monocytogenes* was not detected in 30 sampes, 15 sandwiches (37.5%) and 15 sushi (37.5%) samples. These reuslts are in accordance to Hulankova and Furmancikova (2022) who evaluated the microbiological quality of 135 samples of various sushi types (maki, nigiri, pickled ginger, wasabi paste) purchased at retail, sushi kiosks and restaurants in the Czech Republic and did not detect *L. monocytogenes*. Similary, Miya et al. (2010) did not detect *L. monocytogenes* in 36 samples of sushi in Japan. Interestingly, the first reported food-borne listeriosis outbreak in Japan, occurred in 2001, caused by contaminated cheese (Makino et al., 2005) and this outbreak was detected from routine monitoring in the cheese manufacturing plant. Japan has a unique diet, comprising large quantities of raw RTE seafood, such as sashimi and sushi. Handa et al. (2005) revealed that minced tuna and fish roe products had high contamination rates (14.3% for minced tuna and 10.0 to 11.4% for fish roe products). Our results are in accordance to results obtained by CFS (2022) who examined 100 sandwich samples containing high-risk ingredients (i.e. eggs, cheeses, hams, fresh produce, etc.) and found that microbiological quality was satisfactory. Alegria et al. (2021) evaluated the microbiological quality and safety of 62 samples take-away ready-to-eat sushi meals from different origins (restaurants and hypermarket) in Lisbon, Portugal. All samples were considered to have satisfactory microbiological level for *Salmonella* spp. and *Listeria monocytogenes* which is in accordance to our results. Significant differences were observed between hypermarket and restaurant samples with typical sushi restaurant samples presenting signifficantly ($p < 0.05$) more favourable results when compared to hypermarket samples (Alegria et al., 2021). Contrary to our results are the results from extensively analyses of Szymczak et al. (2020) in Poland which covered 913 samples, including 650 RTE foods: amoung them sandwiches (10), fish products (45), sushi (40). Their results showed that almost 18% of the RTE foods failed to meet the zero tolerance limit for *Listeria*, which means they should not be allowed for retail. *L. monocytogenes* was isolated from 13.5% of the samples, with counts of 10–100 CFU/g noted in half of them. Products with meat and dairy ingredients, fish products, sandwiches, sprouts and sushi, were at the highest statistically significant risk of *L. monocytogenes* contamination (Szymczak et al., 2020).

Among the frozen dairy desserts, ice cream is certainly one of the most popular and is consumed worldwide especially in children population. Although ice cream consumption is typically seasonal during warm months, 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).

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) (Kanbakan et al., 2004; Nalbone et al., 2022) and long storage duration (Bell and Kyriakides, 1998).

Our results showed that out of 10 ice creams samples was not detected presence of *Salmonella* spp. Nalbone et al. (2022) conducted an examination of 200 samples of milk-based industrial ice cream, with and without inclusions, were purchased at different mass-market retailers in Italy. The authors reported unsatisfactory loads obtained for two and nine samples as regarded the aerobic colonies and Enterobacteriaceae, respectively. *L. monocytogenes* was detected in 16 samples, and in three of them, the loads exceeded the legal limit of acceptability (≤100 CFU/g) during marketing. No unsatisfactory loads were obtained for coagulase-positive staphylococci and no *Salmonella* spp. was detected. Our results are in accordance to these due to abscence of *Salmonella* spp. in ice cream. We did not examined the presence of *L. monocytogenes*, but similar to our results were for Enterobacteriaceae. Prevously reported results by Savić Radovanović et al. (2023) showed that out of 40 ice cream samples from catering facilities, 4 (10%) did not meet the hygiene criteria of the production process, as the presence of Enterobacteriaceae higher 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 *Salmonella* spp.

In the Chech Republic, Food Inspection Authority has found an exceptionally high percentage of non-compliant ice cream and beverage ice in 2022, showing that more than half of the ice cream and drinking ice in the Czech Republic does not meet the minimum health and hygiene criteria due 56% of ice cream and 75% of beverage ice considered unsatisfactory. They evaluated 109 ice cream samples in the laboratory, and 61 of them (56%) did not meet hygiene limits. Laboratory analysis of the ice cream samples confirmed a number of Enterobacteriaceae colonies above the limits. The main reasons for these results were non-compliance with production hygiene, inadequate sanitation of equipment, and non-compliance with the production procedure for preparing ice cream. The percentage of non-compliant samples found in 2022 was the highest in previous six years. Comparing to these results, our values were less, but the number of samples was small.

Ice cream is a complex food matrix, and a comprehensive approach to the whole production system is required to ensure high standards of quality and safety (Nalbone et al., 2022).

Conclusion

The examined sendwich and sushi samples were safe for consumption. The obtained results indicate the improvement of hygienic practices and the application of the HACCP system in the production of ready-to-eat-food. In addition, the epidemiological data show that *L. monocytogenes* in not cited as the cause of infection after consuming in the territory of the city of Belgrade.

Out of 10 samples of ice cream, 3 did not meet the hygiene criteria due to presence of Enterobacteriacae.

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DEVELOPMENT OF THE KNOWLEDGE BASE FOR APPLICATIONS OF INFORMATION-EXPERT SYSTEMS IN THE FIELD OF FOOD TECHNOLOGY

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Abstract: This paper presents a comparative analysis of global (ISO) and local (SRPS) Knowledge Sources (KS) in PDCA (Plan – Do – Check – Act) loop quality, with the ability to monitor innovation intensity in the standardized fields. The study refers to fields of the first and second levels of International Classification Standards (ICS) grouped in clusters of innovation. The paper focuses on the latest trends in the KS, trend lines of certain standardized fields, and intensity of innovation in the field of Food technology (ICS1 = 67) and sub-field Beverages (ICS2 = 67.160). The aim is to monitor the intensity of knowledge innovation, trends, KS and update the Knowledge Base (KB) for applications of Information-Expert System (IES) in ICS fields and quality improvement on the standardization platform.

Keywords: application, beverages, Food technology, Information-Expert System (IES), Knowledge Base (KB)

Introduction

Knowledge in the education process often requires significant expenses. Therefore, establishing the mechanism or model of knowledge that will be applied in complex processes bears particular significance. However, the observation and implementation of international (ISO) and local standards (SRPS) are necessary both in education and business processes (ISO, Standards Catalogue ICS). The creation of a Knowledge Base (KB) provides automation solutions to the problem. Knowledge modeling forges a path toward the desired Information-Expert System (IES) in the PDCA loop (Micic et al., 2013).

The research gives insight into the creation of KB for Knowledge Base System (KBS) in the standardized fields/sub-fields, according to the influencing factors for knowledge innovation, viewed from various perspectives. Development of the KB in the field of Food technology can be realized to

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develop IES, starting from Knowledge Source (KS). Based on the defined comparative indicators, such as the index of quality (Iq) and index of value (Iv), innovations are set in the observed knowledge domains (DK).

According to the International Classification of Standards (ICS), all standardized fields are observed (ICS1 = 01, 03 to 99), (ISO, Standards Catalogue ICS, 2024). One of the fields is Food technology (ICS1 = 67) and one of the sub-fields of Food technology is Beverages (ICS2 = 67.160). This sub-field includes the product with the most potential on the market – non-alcoholic beer with *Ganoderma lucidum* extract (Castro-Muñoz, 2019). Strong alcoholic beverages are obtained by alcoholic fermentation from plant raw materials containing sugar or starch, or they are obtained by mixing extracts, essences, and water with ethanol. According to the ethanol content, they can be divided into weak (beer, wine, sake), medium strength (vermouth, prosecco, cherry, amaro, liqueurs), and strong (brandy, whiskey, vodka, gin, rum, cognac).

According to the production process, there are differences between alcoholic beverages that are produced without the application of distillation and those that are produced with the application of distillation beer and wine are produced without applying distillation, and they contain 5 to 15% vol ethanol (Pantović et al., 2020). The application of the beverages produced is judged by the consumers to have new, pleasant, and interesting tastes and color, or good sensory characteristics (Wasser, 2011). The activities and the initial hypotheses in this paper are analyzed in the PDCA concept, through the questions:

1) Plan – Do phases – Is it possible to define comparative indicators (indices) of all ICS fields, to update KB in fields of Food technology and
2) Check – Act phase – Is it possible to define clustering indices of innovation intensity to monitor KS trends of Food technology on the ICS platform?

Standardized knowledge sources in the field of Food technology

Nowadays, the educational process in most higher education institutions also relies on innovative technologies. Regardless of the field in which IES is applied, the use of IT standards is necessary. Standardization is necessary at both levels, global (International Standards, ISO – International Organization for Standardization) and local (National Standards – SRPS). Standardization refers to the connection of knowledge that would lead to the identification of potential differences and the establishment of measures for improvement (Micić and Ružičić, 2016). Innovations (ISO and SRPS) of all fields of the ICS1 classification (time series of KS on 1st January 2015) shows that the weekly innovation cluster (Ii/t = 3, for $50 < \Delta KS_{DK/t} \leq 250$) belongs to a large number of fields, Figure 1 (Ružičić, 2018). Figure 1 shows 40 hierarchically organized fields of standardization covered by the ICS platform. ICS is a hierarchical classification consisting of three levels (ISS, 2023).

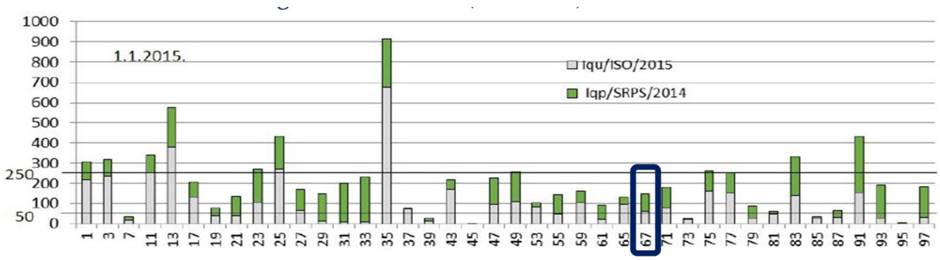

Figure 1. Analysis (ISO – SRPS) of the innovations of the weekly cluster

Materials and methods

The paper used methods of web research, statistical methods, multicriteria analysis, clustering, and surveying. Data were collected from the website of the International Organization for Standardization (ISO, Standards Catalogue ICS) and the National Institute for Standardization (ISS, 2023). The selection and analysis of data have been completed in the form of clustering and determining the level of innovation. Creating trends of KS is followed by mathematical relations (Ružičić and Micić, 2017). Based on $Iqu_{/ISO}$ and $Iqp/_{SRPS}$ and innovativeness intensity Ii/t, it is determined to which innovativeness cluster a field belongs. Based on the frequent innovations expressed by quantities and values of KB units, clustering is performed according to ICS fields (Micić and Ružičić, 2016). The intensity of innovation is viewed according to the rel. (1).

$\Delta KS_{DK/t} = Iqu_{/ISO/t} + Iqp_{/SRPS/t-1}$ (1)

If: $Ii/t > 250$, innovations are daily – daily cluster of innovation (2.1)

$50 < \Delta KS_{DK/t} \leq 250$ – cluster weekly innovation (2.2)

$12 < \Delta KS_{DK/t} \leq 50$ – cluster monthly innovation (2.3)

$0 < \Delta KS_{DK/t} \leq 12$ – cluster yearly innovation (2.4)

$\Delta KS_{DK/t} = 0$ – no innovation. (2.5)

The questionnaire was organized to identify the level of knowledge about the application of IES when teaching students of the Food Technology Study program at the Academic Studies of Faculty of Agriculture in Čačak and the Information Technology Study program at the Academic Studies Faculty of Technical Sciences Čačak, the University of Kragujevac. The research it was conducted during the winter semester of the 2023/2024 school year, in December 2023, participated 52 students in all years of study. The research tends to investigate whether the IES implementation in the educational process has a positive impact on gaining knowledge and motivating an increase of students and users in the field of Food technology, and sub-field Beverages.

Results and discussion

The analysis includes monitoring the frequency of innovativeness in the fields of knowledge and its sources, trends, knowledge of each expert as well as KB updating (Table 1), (ISO, Standards Catalogue ICS). The first level (ICS1) covers 40 fields of activity in standardization. According to relation (2.2) field Food technology is a cluster of weekly innovation ($\Delta KS_{ICS1=67/} = 144$), Table 1.

Table 1. Standardized fields cluster of weekly innovation, ranking list from

	ICS1	**$\Delta KS_{/2013}$**	**Standardization Fileds**
1	71	170	Chemical technology
2	67	144	Food technology
3	65	117	Agriculture

The field of Food technology (ICS1=67) has the next sub-fields of ICS2 (ISO, Standards Catalogue ICS, 2024): 67.020 Processes in the food industry, 67.040 Food products in general, 67.050 General methods of tests and analysis for food, 67.060 Cereals, pulses and derived products, 67.080 Fruits. Vegetables, 67.100 Milk and milk products, 67.120 Meat, meat products and other animal produce, 67.140 Tea. Coffee. Cocoa, 67.160 Beverages, 67.180 Sugar. Sugar products. Starch, 67.190 Chocolate 67.200 Edible oils and fats. Oilseeds, 67.220 Spices and condiments. Food additives, 67.230 Prepackaged and prepared foods, 67.240 Sensory analysis 67.250 Materials and articles in contact with foodstuffs, 67.260 Plants and equipment for the food industry. In the example of the field of Food technology (ICS1 = 67), the creation of a hierarchy of objects (framework) for the formation of rules and the initial KB of the model was demonstrated (Figure 2).

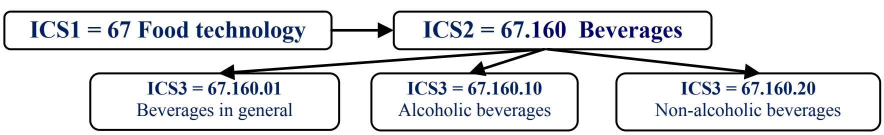

Figure 2. Creation of objects/framework for KB of IES – Food technology

The results of the ICS fields (ISO and SRPS) have been graphically presented both through the review and weekly cluster which belongs to Food technology on the ICS platform (Figure 1, Table 1, relations (1), (2.1)–(2.5)), but the results of research on the need implementation IES in the educational process (Ružičić, 2021), are presented in Table 2. The results show that more than half of the respondents (54.17%) think the IES application ensures more efficient knowledge transfer. Discussion on the PDCA concept:

- (P) Resource Planning for Knowledge Innovation (daily, weekly, or monthly)
- (D) Update of database and KB in Food technology and sub-fields
- (C) Defining clusters according to innovativeness in Food technology
- (A) Monitoring innovation of Food technology for knowledge improvement.

Table 2. Results of the conducted research

Questions	**Answers**		
	Yes (%)	**No (%)**	**I don't know (%)**
1. Do you think that the motivation of the user to apply the innovative Information-Expert System (IES) to enable more efficient knowledge transfer in the fields of Food technology?	69.17	19.72	11.11
2. Does the user's ability to apply IES affect the understanding of the material and the student's satisfaction?	81.22	9.41	9.37
3. Using IES for the clear and precise presentation of the topics in the fields of Food technology and requirements by the users.	86.21	2.59	11.20
4. The IES application is intended for additional explanations and to help students promote their motivation and satisfaction.	90.21	4.34	5.45
5. Presented rules of the knowledge base (KB) to ensure further development and application of IES in special fields such as alcoholic beverages.	76.58	5.22	18.2
6. The IES application ensures the improvement of knowledge in the field of Food technology.	54.17	30.56	15.27

Conclusion

The results of the research show that the IES application ensures the improvement of knowledge in the field of Food technology in the educational process since it positively affects users as well as knowledge acquisition and their performance. The results of the analysis of innovation of the KB and KBS of the local (SRPS) platform utilizing the PDCA/t concept led to the achievement of the objectives of excellence in practice. Based on the analysis of the results, the conclusions can be inferred in PDCA loop quality or entirety:

1. (P–D) Based on $Iqu_{/ISO}$ and $Iqp_{/SRPS}$ and Ii/t it is determined which cluster of innovativeness belongs ICS field and
2. (C–A) It is possible to monitor knowledge source trends of fields of Food technology on the standardization platform, with the purpose of develop IES.

Acknowledgement

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THE INFLUENCE OF PRODUCTION TECHNOLOGY ON THE OVERALL QUALITY OF "VISOČKI SUDŽUK"

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Abstract: The aim of the research was to investigate the impact of production technology on the quality parameters of "Visočki sudžuk." Additionally, the research aimed to examine differences in the quality of "Visočki sudžuk" among various manufacturers within both production technologies. The study found a higher average content of fat, protein, and acidi values in samples from industrial production compared to those from traditional production. Samples from traditional production were characterized by average moisture content, NaCl, ash, aw values, pH, and peroxide number. Differences in the quality of samples from traditional and industrial production, as well as among different manufacturers within both production methods, were identified and found to be statistically significant.

Keywords: quality, production technology, traditional product, "Visočki sudžuk".

Introduction

"Visočki sudžuk" is a traditional Bosnian and Herzegovinian product that has been produced in the Visoko municipality for many years. According to the Rulebook on Minced Meat, Semi-Finished Products, and Meat Products (Official Gazette of BiH No. 82/13), sudžuk is a product made from minced beef, beef fat tissue, kitchen salt or its substitutes, sugar, additives, spices or spice extracts, and starter cultures. It falls under the category of fermented dry cured sausages. The meat protein content in the product must not be less than 16%, and the relative content of connective tissue protein in meat proteins (collagen content) must not exceed 20%. "Visočki sudžuk" is classified as an „unprotected" durable sausage. It is produced based on a manufacturing specification, which must include the product name, producer's name and address, information about the type and quantity of additives and additional ingredients used, product shelf life, and storage conditions (Brkanić, 2019). In traditional production of "Visočki sudžuk," usually meat of the first and second class is used. The meat is prepared and minced

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to obtain so-called minced meat. After adding spices and salt, the minced meat is mixed to create the filling, which is then stuffed into natural intestines that have been cleaned and prepared for use. Following this process, the sausage undergoes drying and smoking at a low heat to prevent rapid external drying while ensuring the interior does not remain raw. With increased market demands and a higher demand for this product, some artisanal producers are adopting an industrial character in terms of production volume. Industrial production of sudzuk introduces certain modifications, diminishing the significance of traditional production and ultimately negatively affecting the product's quality. Although many industrial producers may use traditional production methods or at least conduct drying and smoking in traditional smokehouses, differences in quality are evident. Discrepancies in product quality between traditional and industrial production result from the use of different raw material qualities, amounts of fat tissue, types of intestines, additives, lengths, and conditions of individual production phases. Uncontrolled smoking in traditional smokehouses can negatively impact the quality of the product. Smoking gives special colour, taste and aroma to food, and enhances preservation due to its dehydrating, bactericidal and antioxidant properties (Roseiro et al., 2011; Puljić et al., 2019; Škaljac et al., 2019). However, in addi - tion to the positive effect of the smoking process and a large number of useful compounds that are created by burning wood, harmful components are also created, which include polycyclic aromatic hydrocarbons (PAHs) (Begić et al., 2023). Numerous studies have been conducted to investigate sudžuk quality concerning various factors that directly or indirectly influence its quality (Čaušević and Smajić, 1985; Tupajić, 1991; Gajić, 2000; Sinanović et al., 2005; Operta and Smajić, 2006; Operta et al., 2008; Operta, 2008; Kozačinski et al., 2008; Operta et al., 2012; Operta et al., 2012a; Operta and Smajić, 2012; Čengić et al., 2008; Vukašinović et al., 2012; Kurćubić et al., 2016; Ganić et al., 2018; Operta, 2018; Jahić and Pračić, 2018; Dučić et al., 2018; Brkanić, 2019; Meša, 2020; Kaplan, 2020; Jahić and Rekanović, 2021; Junuzović et al., 2021; Omanović, 2022). The results of these studies, including the present one, form the basis for protecting this traditional Bosnian-Herzegovinian product.

Materials and methods

Materials. For the research, ten samples of "Visočki sudžuk" from traditional and industrial production were used. Five samples of "Visočki sudžuk" from different producers were taken from each production. All samples were purchased in the municipality of Visoko.

Chemical analysis. Fat content was determined by BAS ISO 1443:2007, protein content by BAS ISO 937:2007, moisture content by BAS ISO 1442:2007, ash content by ISO 936:2007, pH was measured using pH meter (FiveGo™ F2, Mettler Toledo, Switzerland), and aw value of the samples was measured using an aw meter (LabSwift – aw, Novasina, Switzerland). Determination of NaCl was performed by Mohr titration using 0.1 M $AgNO_3$ and 5% potassium chromate as an indicator (E. K. 8045, JUS., 1993). Acid value (AV) was used as an indicator of lipolysis. The acid value was determined according to BAS EN ISO 660:2020 method and expressed as mg KOH/g fat. The level of lipid oxidation was assessed by the determination of peroxide value. Peroxide value (PV) was determined according to BAS EN ISO 3960:2017 method and expressed as mmol/kg.

Statistical analyses. All determinations (chemical parameters, indicators of lipolysis and lipid oxidation) were carried out in triplicate, and data were reported as mean ± standard deviation. Influence of production technologies and types of producers was tested using two-way ANOVA, and significant differences ($p<0.05$) were calculated using Tukey test. Statistical analyses were performed using Past software 3.15 (Hammer et al., 2001).

Results and discussion

The results of testing chemical parameters and indicators of lipolysis and lipid oxidation in samples of "Visočki sudžuk" from traditional and industrial production are shown in table 1. A two-way analysis of variance revealed a statistically significant influence of experimental factors, production technology, and types of producers, as well as the interaction between factors on the values of the examined chemical parameters and indicators of lipolysis and lipid oxidation in "Visočki sudzžuk" samples ($p<0.05$). The fat content ranged from 24.56% to 44.73% in samples from industrial production and from 23.68% to 41.26% in samples from traditional production. On average, a higher fat content was found in samples from industrial (35.55%) compared to samples from traditional production (31.81%). Similar fat content values in "Visočki sudžuk" samples from traditional production are reported by Ganić et al. (2018) (31.59%), Brkanić (2019) (30.47-33.22%), Meša (2020) (32.02-33.77%), while Kaplan (2020) (28.80-55.73%) reported higher values in his research. These fat content values align with the results of studies by Operta et al. (2012), Operta et al. (2012a), Operta et al. (2008), Operta (2008), Operta and Smajić (2012), Omanović (2022), and Gajić (2000), indicating fat content in sudžuk ranging from 23.86% to 43.98%. From Table 1., it can be observed that the protein content in the examined samples ranged from 17.26% to 32.96%. In accordance to the previously mentioned, sudžuk is a nutritionally valuable product due to its high protein content. All tested samples comply with the provisions of the Regulation on Minced Meat, Semi-Finished Products, and Meat Products (Official Gazette of BiH No. 82/13) regarding protein content, as the recorded values exceeded 16%.

Table 1. The values of chemical indicators of quality and indicators of lipolysis and lipid oxidation of "Visočki sudžuk" produced on industrially and traditional manner.

Parameter	**Industrial production**				
	P1	**P2**	**P3**	**P4**	**P5**
Fat content (%)	29,54^{Aa}±0,02	38,59^{Ab}±0,08	24,56^{Ac}±0,19	44,73^{Ad}±0,22	40,35^{Ae}±0,41
Protein content (%)	26,02^{Aa}±0,23	25,84^{Aa}±0,38	32,96^{Ab}±0,01	27,18^{Ac}±0,16	30,87^{Ad}±0,22
Moisture content (%)	38,24^{Aa}±0,23	30,35^{Ab}±0,32	33,06^{Ac}±0,23	22,08^{Ad}±0,12	22,99^{Ad}±0,50
NaCl content (%)	4,12^{Aa}±0,00	3,79^{Ab}±0,00	5,38^{Ac}±0,04	4,63^{Ad}±0,01	4,45^{Ad}±0,04
Ash content (%)	5,77^{Aa}±0,08	4,70^{Ab}±0,06	6,43^{Ac}±0,07	5,63^{Aa}±0,01	5,66^{Aa}±0,18
aw value	0,869^{Aa}±0,00	0,855^{Ab}±0,00	0,759^{Ac}±0,00	0,747^{Ad}±0,00	0,753^{Ad}±0,00
pH value	5,02^{Aa}±0,01	4,98^{Ab}±0,01	5,01^{Aab}±0,01	4,79^{Ac}±0,00	4,77^{Ac}±0,01
Acid value (mg KOH g^{-1})	9,82^{Aa}±0,13	13,28^{Ab}±0,26	16,87^{Ac}±0,21	10,16^{Aa}±0,08	8,12^{Ad}±0,11
Peroxide value (mmol kg^{-1})	1,00^{Aa}±0,00	1,99^{Ab}±0,01	1,50^{Ac}±0,00	0,88^{Aa}±0,18	0,38^{Ad}±0,18
Parameter	**Traditional production**				
	P1	**P2**	**P3**	**P4**	**P5**
Fat content (%)	30,81^{Ba}±0,19	26,65^{Bb}±0,29	36,67^{Bc}±0,42	23,68^{Bd}±0,23	41,26^{Ae}±0,17
Protein content (%)	26,21^{Aa}±0,18	32,11^{Bb}±0,53	17,26^{Bc}±0,25	30,59^{Bd}±0,18	30,30^{Ad}±0,27
Moisture content (%)	37,98^{Aa}±0,10	33,72^{Ab}±0,04	38,60^{Ba}±0,19	39,75^{Bc}±0,17	21,86^{Bd}±0,15
NaCl content (%)	4,09^{Aa}±0,03	5,34^{Bb}±0,06	5,69^{Bc}±0,02	4,11^{Ba}±0,02	5,19^{Bd}±0,02
Ash content (%)	4,79^{Ba}±0,15	6,44^{Bb}±0,16	7,51^{Bc}±0,06	5,40^{Ad}±0,02	6,20^{Bb}±0,05
a_w value	0,868^{Aa}±0,00	0,850^{Ab}±0,00	0,839^{Bc}±0,00	0,872^{Ba}±0,00	0,734^{Bd}±0,00
pH value	5,08^{Ba}±0,02	5,41^{Bb}±0,01	4,62^{Bc}±0,01	5,22^{Bd}±0,01	5,19^{Bd}±0,01
Acid value (mg KOH g^{-1})	8,29^{Ba}±0,17	5,96^{Bb}±0,00	15,75^{Bc}±0,21	6,91^{Bd}±0,02	9,83^{Be}±0,02
Peroxide value (mmol kg^{-1})	1,49^{Ba}±0,01	0,62^{Bb}±0,18	1,99^{Bc}±0,01	1,47^{Ba}±0,00	1,38^{Ba}±0,18

P1-P5 Different producers of "Visočki sudžuk"; a-e Different lowercase letters in the columns indicate statistically significant differences between samples of "Visočki sudžuk" produced by different producer; A-B Different capital letters in the rows indicate statistically significant differences between the samples of "Visočki sudžuk" produced in industrial and traditional conditions.

Samples from industrial production had a higher average protein content (28.57%) compared to samples from traditional production (27.29%). These protein content values align with the results of previous research conducted on "Visočki sudžuk," as reported by Ganić et al. (2018) (26.76%), Brkanić (2019) (18.01-23.95%), Meša (2020) (22.32-24.97%), while they are higher compared to values reported by Kaplan (2020) (14.68-26.40%). In previous quality studies of sudžuk, protein content ranged from 20.60% to 33.40%, as reported by Gajić (2000), Operta (2008), Operta et al. (2008), Operta and Smajić (2012), Operta et al. (2012), Operta et al. (2012a), Vukašinović et al. (2012), Kurćubić et al. (2016), Jahić and Pračić (2018), and Junuzović et al. (2021), which falls within the content range established in this study. The moisture content ranged from 21.86% to 39.75%. Samples from traditional production were characterized by higher moisture content (34.28%) compared to samples from industrial production (29.34%). These results are consistent with the findings reported by Gajić (2000), Kozačinski et al. (2008), Operta (2008), Operta et al. (2008), Operta et al. (2012), Vukašinović et al. (2012), Operta et al. (2012a), Dučić et al. (2018), Ganić et al. (2018), Brkanić (2019), Meša (2020), Junuzović et al. (2021), and Omanović (2022). The NaCl content in "Visočki sudžuk" samples ranged from 3.79% to 5.69%. On average, a higher NaCl content was recorded in samples from traditional production (4.88%) compared to samples from industrial production (4.47%). The smallest NaCl content was in sample P2 from industrial production (3.79%), while the largest was in sample P3 from traditional production (5.69%). Differences in NaCl content between traditional and industrial production, as well as between different producers within production, were statistically significant. The NaCl content in samples from traditional production is consistent with the results reported by Ganić et al., (2018) (5.27%), Brkanić (2019) (4.43-5.92%), Kaplan (2020) (3.80-5.61%), and Meša (2020) (4.15-5.16%) in their research. Operta and Smajić (2006) (8.33%) and Jahić and Pračić (2018) (6.24%) reported higher NaCl content in homemade Bosnian sudžuk. The recorded NaCl content in this study aligns with the results reported by Tupajić (1991), Kozačinski et al. (2008), Operta and Smajić (2012), Operta et al. (2012), Operta et al. (2012a), Kurćubić et al. (2016), Jahić and Rekanović (2021), and Junuzović et al. (2021) for sudžuk. Samples of sudžuk from industrial production had lower NaCl content in the studies by Operta and Smajić (2006), Dučić et al. (2018), and Omanović (2022), compared to the samples in this study. The ash content in "Visočki sudžuk" samples was determined in the range of 4.70% to 7.51%. On average, samples from traditional production had higher ash content (6.07%) compared to samples from industrial production (5.64%). The highest ash content was found in samples P3 from both production types (7.51% - traditional and 6.43% - industrial). These ash content values for samples from traditional production align with the results of studies reported by Ganić et al., (2018) (6.29%), Brkanić (2019) (5.56-6.38%), Kaplan (2020) (4.53-6.45%), and Meša (2020) (5.06-5.98%). The results of ash content determination in various sudžuk variants recorded by Operta et al. (2012), Operta et al. (2012a), and Jahić and Rekanović (2021) are consistent with the results recorded in this study. Lower ash content in sudžuk samples from traditional and industrial production is reported by Tupajić (1991), Gajić (2000), Operta and Smajić (2012), Kurćubić et al. (2016), Dučić et al. (2018), Junuzović et al., (2021), and Omanović (2022), while higher values are reported by Jahić and Pračić (2018). The values of water activity (a_w) in the examined samples ranged from 0.734 to 0.872, while pH values ranged from 4.62 to 5.41. On average, higher values of these parameters were characteristic of samples from traditional production (a_w 0.833 and pH 5.10) compared to samples from industrial production (a_w 0.796 and pH 4.91). The a_w and pH values determined in this study in "Visočki sudžuk" samples are consistent with the results of previous research on sudžuk, indicating a_w values from 0.750 to 0.881 and pH values from 4.57 to 5.44 (Kozačinski et al., 2008; Operta et al., 2012a; Kurćubić et al., 2016; Dučić et al., 2018; Ganić et al., 2018; Brkanić, 2019; Kaplan, 2020; Junuzović et al., 2021; and Omanović, 2022;). Lipolytic and oxidative changes in sudžuk have not been studied so far. The values of acidity in the examined samples ranged from 6.91 to 16.87 mg KOH g^{-1}. On average, higher values were found in samples from industrial production (11.65 mg KOH g^{-1}) compared to samples from traditional production (9.35 mg KOH g^{-1}). Samples from industrial production were characterized by a higher average fat content, resulting in more intense lipolytic changes, but, they did not exhibit a higher moisture content, as the activity of lipolytic enzymes increases with the moisture content. Also, the content of n-3 fatty acids increases the sensitivity to lipolytic changes, which may be another reason for these results. The sample P3 from industrial production had the highest acid value (16.87 mg KOH g^{-1}), and it had the lowest fat content (24.56%) compared to other samples from this production. The reason for these results is probably the higher content of more stable saturated fatty acids. In comparison, Glisić (2019) reported lower acid values ranging from 0.74 to 1.47 mg KOH g^{-1} for fermented sausages. Peroxide values were recorded in the range of 0.38-1.99 mmol O_2 kg^{-1}. The highest peroxide values were found in samples P3 from traditional production and P2 from industrial production (1.99 mmol O_2 kg^{-1}). On average, higher peroxide values were recorded in samples from traditional production (1.39 mmol O_2 kg^{-1}) compared to samples from industrial production (1.15 mmol O_2

kg^{-1}). This trend aligns with the findings reported by Kurćubić and Vesković-Moračanin (2017) in their study on the quality of beef prosciutto. Similar to this research, the authors found higher peroxide values in samples from traditional production (2.00 mmol O_2 kg^{-1}) compared to samples from industrial production (1.50 mmol O_2 kg^{-1}). They explained this phenomenon by the stronger action of pro-oxidative factors on samples from traditional production, as they were not vacuum-sealed, unlike samples from industrial production, which were vacuum-sealed. According to the results mentioned, Glisić (2019) reported lower peroxide values for fermented sausages at the end of production, ranging from 0.06 to 0.31 mmol kg^{-1}.

Conclusion

This study aimed to provide information about the quality of "Visočki sudžuk" from traditional and industrial production, as well as the quality variation among different producers. The most significant differences were observed in fat content, ranging from 23.68% to 44.73%, and moisture content, ranging from 21.86% to 38.24%, depending on the production method and producer. Samples from traditional production were characterized by higher moisture content, resulting in higher water activity values. Additionally, samples from traditional production had higher levels of NaCl, ash, and pH values. More intense lipolytic changes were noted in samples from industrial production, while more pronounced oxidative changes were observed in samples from traditional production. To further explain these changes, future research should focus on determining the fatty acid composition of "Visočki sudžuk."

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FORMATION OF HYDROXYMETHYLFURFURAL DURING THE INDUSTRIAL PRODUCTION OF PLUM PEKMEZ

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Abstract: It was analyzed the industrial plum pekmez production: blanching, mashing, cooking blanched plum pulp with invert sugar solution and caramel, final heat treatment, cooling pekmez and surface treatment. The aim was to determine the critical stages in which hydroxymethylfurfural (HMF) forms. HMF is synthesized by the thermal sugar decomposition or as one product of the Maillard reaction and can be an indicator of the thermal degree of food processing. Also, HMF is a quality parameter of food due to its potentially harmful effect on consumer health. The cooking blanched plum pulp was a critical stage when the HMF content was significant increased.

Keywords: plum, pekmez, hydroxymethylfurfural

Introduction

The hydroxymethylfurfural or 5-hydroxymethyl-2furaldehyde (HMF), is a cyclic aldehyde built from a furan ring with an aldehyde and an alcohol functional group. HMF can be formed in various reactions, including Maillard reactions, when the carboxyl group of reducing sugars react with amino acids, peptides or proteins or by the dehydration of hexoses, in an acidic environment (caramelization) during thermal processes (Lee et al, 2019). Data on the impact of HMF on human health vary. There are reports about cytotoxic, genotoxic and carcinogenic effects, but also positive health effects like antioxidant activity, anti-carcinogenic and anti-hypoxic action (Choudhary et al, 2021).

The pekmez is a product with an appropriate thickened consistency, produced by cooking fruit pulp and/or fruit pulp/puree of one or more types of fruit, with or without added sugar with a maximum of up to 25% in relation to the total amount of fruit (Official Gazette, 2015). The applied heat treatment during pekmez production causes changes in primary fruit chemical

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composition and the loss of many naturally presented nutrients (Kim and Padilla-Zakour, 2004), and the formation of potentially harmful by-products such as HMF. The amount of HMF depends on the applied method of processing and storage conditions (Kuşçu and Bulantekin, 2016; Oral et al, 2012). The higher temperature and duration of fruit thermal treatment causes the formation of a larger amount of HMF. It has shown that vacuum evaporation can be used effectively in pekmez production, which prevents HMF formation (Kuşçu and Bulantekin, 2016).

Therefore, the HMF content can be used as an indicator of food thermal processing (Radovanović et al, 2017) and also as a quality parameter (Đurović et al, 2022), with the view that it is considered potentially harmful to human health. The aim of this research was to determine the critical stages in the industrial production of plum pekmez, in which HMF forming.

Materials and methods

Materials

Samples of frozen plums, invert sugar solution, blanched plum pulp, semi-pekmez and final product-pekmez were sampled in the local fruit processing factory during the production of plum pekmez with invert sugar solution. The invert sugar solution was prepared by sucrose hydrolysis with citric acid as a catalyst. All chemicals were p.a., except those of HPLC grade methanol (J. T. Baker, Netherlands) and the HMF standard (Dr. Ehrenstorfer GmbH, Germany).

Production of plum pekmez

The industrial production of pekmez implies a series of related and well-coordinated processes: from the reception of fresh fruit to the final product, with certain specificities (Fig. 1). It used plum fruits mix of the Stanley, Čačanska rodna and Požegača varieties. Defrosted fruits were transported for blanching, 85–90 °C for 15 to 18 min. The heated plum was then transported to a sieve (diameter 3.5 mm), where the stones were separated. This was followed by fine mashing on sieves with diameters of 1.2 mm and 0.6 mm. After mashing plum pulp was cooked at 70 °C and 0.85 bar, until the content of dry matter of 54 to 57% was reached. Halftime through the cooking, invert sugar solution was drawn into the system. Towards the end of the cooking, caramel (E-150d) was added, by first dissolving it in a small amount of pulp, and only after that it is drawn into the steamer and the cooking process continues.

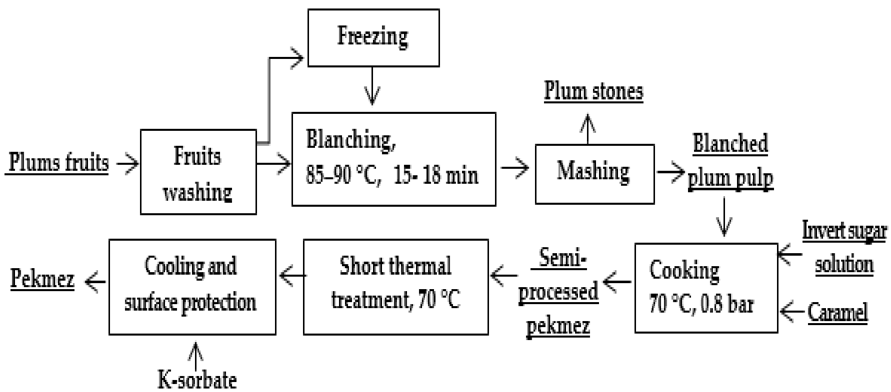

Fig. 1. Scheme of plum pekmez production in local fruit processing factory

The semi-processed pekmez then was heated to 80 °C, and cooled to a temperature of 20 to 25 °C. The cooled pekmez was exposed to surface protection with a 6% solution of potassium sorbate and transported in a refrigerator where it was stored at a temperature of 4 to 6 °C until distribution to the customer.

Determination dry matter

Dry matter was determined by Abbe refractometer (A. Krüss-Optronic, Germany), with a precision of ±0.00. Stones were removed from the defrosted plum fruits, then plums were crushed and homogenized with the liquid phase that was released after defrosting. Other samples did not require special preparation for measurement.

Determination of HMF content

The HMF content was determined by the liquid chromatography –HPLC (Waters, USA), as described by the authors Vorlova et al. (2006). The concentrations of Hmf standards were in the range from 0.015 mg·mL^{-1} to 0.236 mg·mL^{-1}.There were weighted 2±0.001 g samples, dissolved in a small amount of deionized water and 2 mL of Carrez Ⅰ and Carrez Ⅱ solutions were added to each sample and filled up to 50 mL volumetric flasks with deionized water, wellhomogenized and left for 30 minutes to clear. Before injection, each sample was filtered through an ALLPURE NY membrane filter (0.45 μm) and homogenized by a shaker (Ika MS 3 digital, China). Separation of components in the sample was performed on a Spherisorb ODS2 column (4.6 mm×250 mm, 5 μm), at 30 °C. It used an isocratic mode with a mobile phase flow rate of A: water 0.9 $mLmin^{-1}$, B: methanol 0.1 mL□min^{-1}(Binary Waters pump 1525). 20 μL of the standard or sample solution was injected each time (Autosampler 717 plus). The measurement was performed at 285 nm (Dual λ Absorbance Detector 2487). The retention time of the HMF standard peak and HMF in samples that occurred is at 7.7 and 8 min, respectively.

Determination of total acidity in plum

This method is based on the titration of the test sample with NaOH solution in the presence of a phenolphthalein indicator until a persistent light pink color appears (Official Gazette,1983). Total acidity is expressed as malic acid.

Polarimetric determination

A polarimeter POl 1, (Optica, Italy) with sodium lamp (589.44 nm) was used. Solutions of invert sugar were prepared in the concentration range of 0,05 gL^{-1} to 2 $g{\cdot}L^{-1}$ by adding five drops of NH_4OH (conc.25%) in a 100 mL volumetric flask to prevent sugar mutarotation. The specific angle of optical rotation was determined graphically from function: α=f(c) and Biot´s law.

Results and discussion

Properties of plum fruit and invert sugar solution

According to Official Gazette (2021) the minimum content of soluble dry matter for plums used for processing, except for variety Požegača, is 12%, and for Požegača is 14%. A mixture of three varieties: Požegača, Stanley and Čačanska rodna was used for the production of pekmez and the sample of defrosted plum had a dry matter of 14.5% (Table 1). The acidity of plum fruits depends on the variety, climatic, growing and storage conditions. It was analyzed defrosted plum sample, so the acidity can be changed during the freezing process (Van der Sman, 2020).

Table 1. Properties of plum fruit and invert sugar syrup

Defrosted plum		Invert sugar solution		
Soluble dry matter, %	Total acidity (%, w/w)	Dry matter, %	HMF, mgkg^{-1}	[α], °cm^3·g^{-1}·dm^{-1}
14.5±0.0	0.3±0.025	71±0.0	1.55	47.17*

*obtained from the calibration curve: α=f(c), R^2=0.9746

Invert sugar solution was produced in the industry by hydrolysis of sucrose with citric acid at 60 °C and under 0.8 bar. The content of dry matter in a sample of invert sugar solution was in accordance with the Official Gazette (2017), where it is defined as a minimum content of dry matter of 62%. The invert sugar solution was obtained by partial hydrolysis of sucrose, which confirms the specific rotation of the plane of polarized light. The specific rotation of pure sucrose solutions (66.5 °cm3·g^{-1}·dm^{-1}) has decreased (47.17 °cm^3·g^{-1}·dm^{-1}) due to the formation of monosaccharides, whereby fructose turns the plane of polarized light to the left and glucose to the right.

There was recorded the HMF presence in invert sugar solution as a consequence of the carbohydrate breakdown under the thermal treatment in acid media, but the application of lower temperature and pressure (60 °C, 0.8 bar) during hydrolysis probably contributed to the formation of HMF in a small amount, 1.55 mgkg^{-1}.

Formation HMF during plum pekmez production

Comparing the chromatograms of all analyzed samples (Figure 2) and amounts (Table 2) can be observed that the least amount of HMF was formed during the production of the invert sugar solution. It is expected, because the cooking time was the shortest and the temperature during the process was the lowest. A slightly higher content of HMF was observed in the blanched pulp, because the fruits were treated at a significantly higher temperature during blanching in the thermobrick. Although this process takes significantly less time than sucrose hydrolysis, the high temperature and composition of the medium most likely contributed to these results. It was observed that the resulting semi-processed pekmez had a higher content of HMF, due to the effect of longer exposure to high temperature. The continuous addition of blanched pulp during cooking can mask the real content of HMF in semi-processed pekmez.

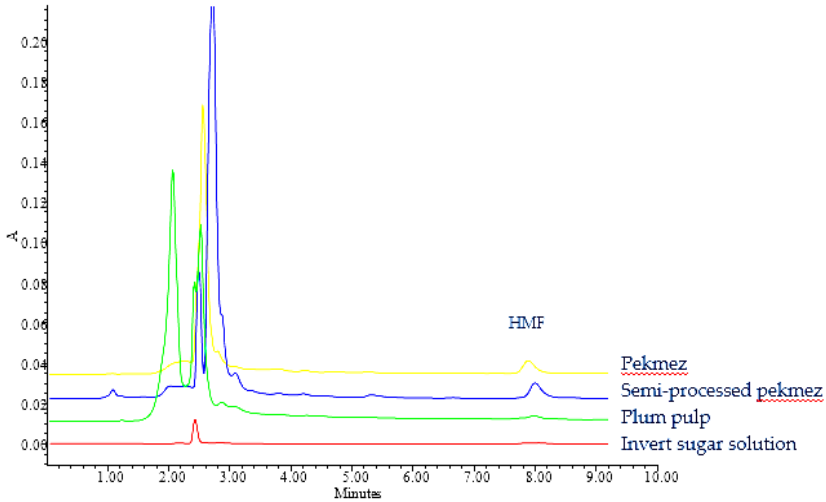

Fig.2. Comparative chromatograms of: invert sugar solution, blanched pulp, semi-processed pekmez and pekmez

The highest content of HMF was determined in the pekmez which is expected, if we take into account that the cooking time was the longest, and that the product was briefly exposed to a slightly higher temperature towards the end of cooking. Mendoza et al (2002) found HMF in all samples of jams, regardless of the pH, sugar or dry matter, from traces to 7.17 mg/100 g product (mean value close to 1.35 mg/100 g product).

The content of dry matter in the pulp (Table 2) was below the optimal values (from 16 to 23%) provided by the recipe, which was probably a consequence of losses during defrosting and the use of plum fruits with an almost low content of dry matter.

Table 2. Content of dry matter and HMF in pulp, semi-product and finished product – pekmez

Parameter	Pulp	Semi-pekmez	Pekmez
Dry matter, %	14.75±0.0	54.25±0.0	57.00±0.0
HMF, $mgkg^{-1}$	5.55	18.10	18.60

Therefore, longer cooking was applied, so that the final product has a needed content of dry matter. In the semi-processed pekmez, the expected significantly higher content of dry matter was observed than in the pulp, which was a consequence of the cooking process lasting several hours.

Conclusion

The industrial production of plum pekmez is a complex process that includes several stages: washing plum fruits, freezing, blanching, separating stones, fine mashing, cooking, cooling and protecting pekmez surface with K-sorbat. The thermal treatment applied during production invert sugar solution and plum pekemez influenced the formation of hydroxymethylfurfural. HMF was observed in invert sugar solution, blanched pulp, semi-processed pekmez and the final product-pekmez. The content of HMF in the invert sugar solution, produced with citric acid, was the lowest due to the sucrose hydrolysis performed at 60 °C and under 0.8 bar. In the blanched plum pulp the HMF was quantified in the amount of 5.5 $mgkg^{-1}$. The cooking blanched plum pulp was a critical stage when the HMF content was increased more than 3 times. Based on the results, it is clear that the HMF is an indicator of the thermal treatment of plums during pekmez production.

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QUALITY OF MINCED MEAT PRODUCTS

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Abstract: Minced meat is a popular and widely consumed food worldwide. The quality of meat products is often associated with microbiological correctness, sustainability, and sensory properties. The quality of minced meat and minced meat products is affected by a variety of factors. The European Union (EU) has established regulations to standardize and enhance the quality of minced meat products. Adherence to these regulations plays a crucial role in meeting the desired nutritional composition and sensory attributes of minced meat products.

Keywords: minced meat, burger, quality, ćevap, chemical composition

Introduction

The consumption of meat varies globally due to cultural differences, traditions, and raw material availability. The FAO reports an increase in global meat consumption due to individual incomes and population growth. Recent changes include increased consumption of pork and poultry, as well as processed meat, reflecting the changing global meat landscape (Godfray et al., 2018). Minced meat is in demand in countries all around the world due to its use as the main raw material in the production of popular food such as hamburgers, sausages, meatballs, and patties in Western countries, as well as Balkan dishes like ćevap and pljeskavica (Setiadi et al., 2022). These types of products are becoming more widely accepted due to their simple preparation and ease of use (Setiadi et al., 2022, Stajić et al., 2021). Additionally, besides semi-finished products and products made entirely from minced meat, there are ready-to-eat meals and frozen meals whose main ingredient is minced meat. Some of these products such as Pasta Bolognese, Quesadillas, Meatballs, Meat Burek, Greek Moussakas, are well-known worldwide (Tomasevic et al., 2023). Nowadays, a wide variety of minced semi-finished products can be found in stores. Many of these products are made from mixed minced meat by combining different types of meat, resulting in improved nutritional characteristics and a well-balanced chemical composition (Tsaregorodtseva, 2021). According to the authors, the estimated world value of the minced meat

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market for 2021 was worth $66.3 billion, and it is expected to increase by more than 3% by the end of 2023 (Tomasevic et al., 2023).

Materials and methods

This review study analyzed scientific papers and articles published between 2014 and 2023 on minced meat quality, using databases like Web of Science, ScienceDirect, Scopus, and Mendeley. Keywords included minced meat and meat preparations, quality parameters, sensory, physical, and chemical characteristics, microbiological quality, and consumption. Quality parameters were classified based on factors like author, year of publication, type of minced meat, usable meat, and heat treatment. Data mainly focused on technological, nutritional, sensory, and microbiological quality parameters.

Results and discussion

The basic chemical composition of minced meat products is determined by the proportion and quality of individual tissues, and additional ingredients used in their production (Lawrie and Ledward, 2006). The quality of a product is significantly influenced by the quality of its raw materials. The meat of older animals has a firmer structure with differentiated connective tissue, where the proportion of fatty tissue varies depending on the method of fattening. The connection between shredded muscle and fat tissue weakens as the proportion of fat tissue increases. External factors such as table salt and molding pressure also affect this connection. Increasing the degree of fragmentation increases the dispersivity of the particles and the share of proteins in the dispersion medium, which affects the plasticity and increases the hydration capacity. One of the most important properties of minced meat is its ability to bind water. Lower quality meat generally has a lower ability to bind water (water binding capacity), because muscle proteins have a greater ability to bind water compared to connective tissue proteins, the proportion of which increases if the quality of the meat is poorer (Polak et al., 2018, Muftić et al., 2020). The fat content is usually adjusted to achieve the desired taste and texture because fats add flavor and juiciness to products (Stajić et al., 2023). The chemical composition of minced meat products can vary depending on the ingredients, the method of preparation, and also on the type of meat, which is shown in Table 1. There are insignificant distinctions in chemical composition between Western minced meat products and traditional Balkan ones, named ćevap.

The European Union (EU) has established special regulations (Regulation EC No. 853/2004), which are harmonized with the Regulation on Food Safety of Serbia (Regulation RS No. 50/2019; 34/2023) regarding fat content and collagen ratio and meat protein in minced meat products due to their non-standard composition and limited sustainability. This regulatory framework was developed to ensure standardization and enhance the quality of minced meat products across the EU market.

Table 1. Chemical composition of minced meat products

Type of meat	Author	Type of product	Moisture %	Protein %	Fat %	Ash %	Cooking loss %	pH
pork	(Bellucci et al., 2021)	patties	71.45	15.28	9.37	2.57	22.53	5.61
	(Foggiaro et al., 2022)	burger	70.45	17.34	10.03	1.9	26.41	5.67
	(Witte et al., 2022)	minced meat	72.73	19.57	8.33	-	28.27	5.62
	(Djekic et al., 2023)	ćevap	65.35	15.53	16.67	-	17.99	6.83
beef	(Leite et al., 2022)	patties	60.92	21.46	12.19	1.5	24.39	6.26
	(Barros et al., 2020)	burger	73.19	19.21	4.42	2.09	22.34	5.58
	(Heck et al., 2017)	burger	62.4	19	17.4	2.5	32.1	6.2
	(Zinina et al., 2020)	minced meat	73.5	16.6	8	-	-	-

Minced meat products are popular due to their appealing sensory qualities. Because of large impact on sensory characteristics, fatty tissue and salt are particularly essential elements in the formulation of minced meat products. Salt influences flavor, shelf-life, and safety, as well as textural attributes, by boosting protein solubilization and extraction, increasing water-holding capacity, and enhancing processing yield and juiciness (Stajić et al., 2021). The appearance, smell and taste of fresh and microbiologically correct minced meat are characteristic of the meat of the animal from which the product was obtained. It has a firm-soft consistency, does not spread and is not sticky. The color varies depending on the species, age and diet of the animal, between light and dark red. The chopped meat is checkered red, interspersed with pieces of white fatty tissue and tendons, again depending on the type of animal (Zinina et al., 2020).

Thermal processing of minced meat results in a color change from red to dark brown due to the Maillard reaction. This process reduces the nutritional value of the meat, with the intensity of taste change varying based on the method, temperature, and duration of treatment. The softness of minced meat products is achieved through grinding, but loss of meat juice during shredding and thermal processing also affects it. Factors like age, musculature properties, and degree of autolysis also impact the softness and sensory properties of minced meat products (James and Yang, 2011). Comparative analysis of minced meat-based products is challenging due to differences in meat types, product characteristics, and preparation methods. Table 2 reveals a significant difference in hardness and chewiness values for the traditional Balkan delicacy, ćevap. This discrepancy can be attributed to the distinct shaping techniques employed in the preparation of ćevap, which vary across the Balkan region.

Table 2. Texture parameters of minced meat products

Type of meat	Author	Type of product	Hardness (N)	Springiness (mm)	Cohesiveness	Gumminess (N)	Chewiness (N×m)
pork	(Bellucci et al., 2021)	patties	128.32	0.78	0.59	75.55	58.69
	(Djekic et al., 2023)	ćevap	35.09	0.89	0.45	15.88	14.19
beef	(Barros et al., 2021)	burger	141.46	0.73	0.55	77.9	56.89
	(Barros et al., 2020)	burger	174.1	0.8	0.62	108.7	87.68

The term quality of meat products is most often associated with microbiological correctness, i.e., sustainability, and sensory properties. Minced meat is a suitable environment for microflora development due to its chemical composition and value. Microorganisms in fresh meat are predominantly from the *Micrococcaceae* family, while *Pseudomonadaceae* prevail during cooling and *Lactobacillus* in vacuum-packed meat. Contamination levels depend on animal conditions before slaughter, slaughter, and primary processing conditions (Vuković and Zorić, 1992).

Each meat product placed on the market must fulfill the food safety requirements explicitly stated by EU Regulation (EU, 2005), which is in line with Serbian law (Serbia, 2010, 2018). According to the regulations, the absence of *Salmonella* spp. in 5 units per 10 grams of minced meat is required for a microbiologically correct product, a limit of 2 in 5 samples between 50 cfu/g and

500 cfu/g for *Escherichia coli* and between 5×10^5 cfu/g and 5×10^6 cfu/g for aerobic colonies. These limits and methods may change based on regulations.

Conclusion

The quality of minced meat and minced meat products is affected by a variety of factors. All the data mentioned in this paper show that the basic composition of ground meat and similar products is very variable. Hygiene is essential for sustaining product quality, and the use of diverse methods and technology can assist enhance quality. As a result, it is critical to identify the best process for producing minced meat and minced meat products in order to assure product quality and safety.

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THE INFLUENCE OF MACERATION TIME ON THE CHEMICAL AND SENSORY CHARACTERISTICS OF WINES FROM THE SERBIAN AUTOCHTHONOUS VARIETY "PROKUPAC"

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Abstract: In this study, the influence of maceration time on the chemical and sensory properties of the red wine grape variety Prokupac (*Vitis viniferae* L.) was investigated. The main focus was on the total phenolic content (TPC) and other chemical components of the wine whose content is influenced by maceration time and other conditions of vinification. The addition of yeast could also influence the properties of the wine, which is why spontaneous and inoculated fermentation were observed. In both cases, maceration lasted 0, 3, 5, 7, 14 and 21 days, respectively. The highest extracted value of TPC was reached on the 12th day during inoculated fermentation (1940.11 mg GAE/L) and on the 21st day during spontaneous fermentation (1793.00 mg GAE/L). Apart from TPC and other chemical components of the wine, these values depend on the wine technology used.

Keywords: phenols, maceration time, Prokupac

Introduction

It is known that winemaking variables can affect the chemical properties of wine. It has been reported that some of the winemaking processes increase the phenolic concentration in red wine and alter wine parameters such as total extract content, ash content, total acidity, color intensity and alcohol content.

It has been shown that contact time with the skins can affect phenolic extraction, but the maximum extraction time is not the same for all grape varieties (Atanackovic et al., 2012), nor for the same variety with different technologies applied. In order to produce good quality wine, the technological characteristics of the predominant grape varieties in Serbia were investigated.

"Prokupac" is a Serbian autochthonous wine variety. It is characterized by less vigorous growth and a good yield. The shoots of Prokupac are developed and strong, with a vertical rise, can be grown on training systems with a small

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trunk. Fertility is good - the lowest buds on the shoot are very fertile, so short pruning in practice ensures an excellent yield (Petrovic et al., 2019). The "Prokupac" grape variety has a very long tradition in red wine production, but has been neglected for decades in favor of international wine varieties known for their potential to produce high-quality wines (Bešlić et al. 2012).

An important step in the production of red wine is the maceration process, the effects of which influence the quality of the wine obtained (Bautista-Ortín et al., 2005; Lisov et al., 2020). The main objective of maceration is the extraction of colourants (anthocyanins and phenolic compounds) from the solid components of the grape. However, this process also affects the sensory profile of the resulting wines, as other compounds such as aroma compounds and precursors, polysaccharides, minerals and other components are released during the maceration process (Francesca et al., 2014). Longer maceration times also ensure the stability of the colourants that contribute to the colour intensity of the wine (Gómez Plaza et al., 2002). Among these, phenolic compounds are gaining increasing interest due to their positive effects (antioxidant, anticancer, cardioprotective, antimicrobial, antiviral and neuroprotective) on human health (Francesca et al., 2014).

In addition, many factors, including the yeasts involved in the fermentation process, can influence the phenolic content of wine (Morata et al., 2019). Indeed, yeast metabolism during fermentation can lead to various metabolic precursors that influence the composition of wine. Some studies indicated that the presence of enzymes produced by the indigenous mycobiota can influence the biochemical reactions of phenolics during fermentation, thus altering their content in wine (Boban et al., 2024).

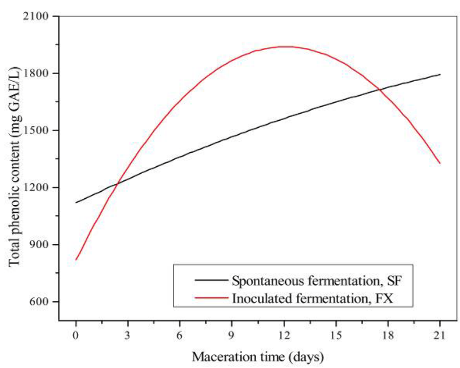
The present study represents the first attempt to investigate the correlation between the autochthonous mycobiota of Prokupac grapes from the viticultural sub-regions of Prokuplje in Serbia and the phenolic composition and physico-chemical parameters of wines produced by both spontaneous fermentation (SF) and inoculation (FX10). The main focus of this research was to determine the optimal vinification conditions for obtaining red wine with the highest possible phenolic content, as well as other wine ingredients that could contribute to high sensory quality.

Materials and methods

The Prokupac grape variety was harvested in a state of technological maturity. Alcoholic fermentation with maceration was carried out using the microvinification method at a temperature of 25°C using the Pigéage system. Two different vinifications were carried out, one spontaneous and one inoculated. The *Saccharomyces cerevisiae* yeast strain (FX10, Laffort, France) in the amount of 20g/hL was used. Sulphur dioxide was added in the form of $K_2S_2O_5$ (10 g/100 kg crushed grapes) during maceration. In both cases, maceration lasted 0, 3, 5, 7, 14 and 21 days, respectively. After alcoholic fermentation and racking, the wines were bottled and stored until analysis. All physico-chemical parameters (total extract content, ash content, total acidity and colour intensity) were determined according to the OIV (Compendium of International Methods of Wine and Must Analysis). The total phenolic content (TPC) of the wine samples was determined according to the Folin–Ciocalteu method (FC) using gallic acid as a standard.

Results and discussion

Two different vinifications were observed (FX10 and SF), and the same six maceration times (0, 3, 5, 7, 14 and 21 days) were observed in both (Graph 1). During spontaneous fermentation, an increase in total phenolic content was observed until the 21st day of maceration. The maximum extracted value during SF was 1793.00 mg GAE/L (Graph 1.). When inoculated with the selected wine yeast FX10, the total phenolic content increased until the 12th day of maceration. This is probably due to the possible adsorption to the yeast cells. The maximum extracted value was 1940.11 mg GAE/L on day 12. The lowest concentration of total phenolic content was found in the control wine samples (must separated immediately after crushing).



Graph 1. TPC in wine samples during 21 day of maceration.

A significant difference was found between the TPC obtained in SF and FX fermentation ($p < 0.05$). Damijanic et al. (2012) analysed the dynamic extraction of phenolic compounds from the Teran grape variety during the 21-day maceration and found the highest content on the 17th day. Kocabey et al. (2016) also demonstrated that the duration of maceration on TPC in Karaoglan wine from the 5th to the 15th day was significantly different. Another study confirmed that a higher TPC content was obtained with increasing maceration duration (Budić-Leto et al., 2008; Lisov et al., 2020).

According to Petrovic et al. (2019), who analysed different clones of Prokupac from Serbia, the total acid content varied between 5.70 g/L and 10.45 g/L, expressed as tartaric acid. This is consistent with our results, but depends almost entirely on weather conditions and pH, which was slightly higher than typical for wine (Table 1.).

Table 1. Physico-chemical parameters of wine samples.

Wine	Total acidity (g/L)	pH	Total extract content (g/L)	Total ash content (g/L)	Colour intensity (CI)	Hue	Proportion of yellow colour (%Y)	Proportion of red colour (%R)	Proportion of blue colour (%B)
SF0	5.78	3.99	21.60	1.78	0.38	0.83	40.58	48.69	10.73
SF3	6.35	3.91	24.61	2.26	0.97	0.65	33.78	51.70	14.52
SF5	5.78	3.97	22.25	2.75	0.90	0.76	35.22	46.62	18.16
SF7	5.28	4.01	22.94	2.80	0.83	0.73	35.42	48.67	15.90
SF14	5.86	4.16	25.40	2.86	0.60	0.81	39.80	49.00	11.20
SF21	4.54	4.18	19.90	2.09	1.11	0.75	35.10	46.77	18.13
FX0	6.68	3.73	21.01	2.80	0.18	2.17	63.19	29.12	7.69
FX3	5.20	4.41	22.24	3.47	0.76	0.77	38.10	49.60	12.30
FX5	4.95	4.00	21.23	2.96	1.06	0.82	38.58	46.82	14.60
FX7	5.03	4.15	20.80	3.35	0.77	0.81	38.63	47.67	13.70
FX14	6.27	3.96	22.08	3.02	1.00	0.85	39.26	46.35	14.39
FX21	5.03	4.17	20.22	2.91	0.50	0.98	44.04	44.85	11.11

The total extract content varied between 19.90 g/L and 25.40 g/L in wines produced by spontaneous fermentation. In inoculated fermentation, the lowest total extract content was measured in wines whose maceration lasted 21 days, which could be attributed to the adsorption of these compounds on the grape solids and lees during the longer contact time. A significant difference in total extract content was found between SF and FX ($p< 0.05$). According to Lakicevic et al. (2020), the total extract content of Prokupac was 16.90 g/L, which is lower than wines analysed in this study. In an earlier study (Glories, 1984), the optimal ratio between the components of red wine colour was reported as %Y:

35, %R: 55 and %B: 10. Our results were almost similar to those reported by Glories.

As far as the sensory characteristics are concerned, the wine whose maceration lasted 7 days and which was used during inoculated fermentation showed the best sensory properties. The colour of the wine was dark ruby red. The aroma was the most complex with pronounced fruitiness and diversity. Rounded taste with nice balance and aftertaste. The SF wine with a maceration period of 14 days showed the best sensory quality. It had a ruby red colour, a characteristic fruity smell of medium intensity and a taste typical of the Prokupac variety.

Conclusion

The present study, in which the effects of maceration time and the comparison between spontaneous and inoculated fermentation were investigated, showed significantly different effects on the total phenolic content and other physico-chemical parameters of the wine. This study offers a perspective for future research into winemaking techniques for obtaining high-quality wines from the Prokupac grape variety.

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DETERMINATION OF HEAVY METALS IN FRESH FISH

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Abstract: Consumption of fish has increased in last 50 years. Fish as a food is changing red meat because it has unsaturated fat and it is the best source of omega 3 fatty acids. Beside it is full of minerals, vitamins and it has high biological value of proteins.The content of heavy metals in the muscle tissue of fish is directly related to the pollution of the water they come from

The analysis of the content of heavy metals was done by the Institute of Public Health of the Federation of Bosnia and Herzegovina.The content of lead (Pb) in the tested samples of fresh fish ranged from 0.0015 to 0.0381 mg/kg. The measured content of cadmium (Cd) in the examined samples was in the range of $3.3*10^{-5}$ to 0.0053 mg/kg. The content of arsenic (As) in the tested samples ranged from 0.0085 to 1.1668 mg/kg. The mercury (Hg) content in the tested samples of fresh fish ranged from 0.0033 to 0.0991 mg/kg, which is within the allowed values prescribed by the Rulebook. It has been statistically proven that there is a significant difference in the measured values of lead, arsenic and cadmium in the samples of sea and freshwater fish.

Aim of this work was to establish do the samples of fresh fish contain concentration of heavy metals more than concentrations prescribed in Rule book about allowed amounts of certain contaminants in food. Thereby ten samples of fresh fish were tested, five samples of marine fish and five samples of freshwater fish. Results showed that all samples of fish satisfy allowed concentration of heavy metals according to the Rule book.

Keywords: Fresh fish (freshwater and marine fish), heavy metals, contaminants

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Introduction

Modern science has clearly defined the mutual conditioning of water contamination and the content of heavy metals in the organisms of its inhabitants, as well as the ways and routes of intake. It is known from the literature that aquatic organisms accumulate elements from water, even those found in traces. There are different mechanisms of accumulation, which depend both on the element that accumulates and on the organism that accumulates them from water, sediment, through food or contact with dispersed particles(Karahmet et al., 2020., Alić et al., 2004).

Fish as a food can be source of heavy metals such as: Cu, Zn, Cd, Hg and Pb. Contaminants in fish are coming uncontrollably from enviroment or they can be rests of testings which people were using in different phases of producing (Isaković et al., 2021). The intake of heavy metals is not always the result of human activity, but is also the cause of adsorption processes that occur due to the presence of natural soil ingredients (Šarkanj et al., 2010).

Intake of heavy metals, such as lead, copper, cadmium can be potentially toxic, and regular monitoring and monitoring of the factors that determine that intake is necessary. The intake of heavy metals is not always the result of human activity, but is also the cause of adsorption processes that occur due to the presence of natural soil ingredients, (Ashraf, 2006, Korjenić, 2008).

It is known from the literature that aquatic organisms accumulate elements from water, even those found in traces. There are different accumulation mechanisms, depending on the elementthat accumulates, as well as from the organism that accumulates them from water, sediment, through food or contact with dispersedparticles (Tomašević et al., 1988).

Also statistic analysis was done with aim of establishing of eventual differences of heavy metals concentrations between individual samples. Analysis showed existence statisticly important differences in content of heavy metals depends on fish species and their origin.

Material and methods

Ten samples were used to test the content of heavy metals in fresh fish. Five samples of marine fish and five samples of freshwater fish. From saltwater fish: blue hake (*Merluccius merluccius*), sardine (*Sardina pilchardus*), mackerel (*Scomber scombrus*), tuna (*Thunnus*) and Gilt-head seabream (*Sparus aurata*) were used, while samples of freshwater fish consisted of brown trout (*Salmo trutta —*

morpha fario), rainbow trout (*Oncorhynchus Mikiss, Linnaeus*), Carp (*Cyprinus Carpio*), Chub (*Leuciscus cephalus, Linnaeus 1758*) and Gibel carp (*Carassius gibelio*). All fish were delivered to the Institute of Public Health of the Federation of Bosnia and Herzegovina, where the analysis of heavy metals in fish was done.

Two methods were used to determine the content of heavy metals in fresh fish. For the determination of mercury, the Method for determining the content of mercury in fish and fish products was used on the direct mercury analyzer AMA 254, while the determination of the content of other metals (Pb, Cd, As) was done according to the Method for determining heavy metals in food by analysis on an atomic absorption spectrometer.

The prepared sample is transported to the weighing room. After weighing, the sample is transferred to the desiccator to the direct mercury analyzer AMA 254. After starting the program, it is necessary to wait 20 minutes for the device to stabilize. Before starting to analyze the sample, it is necessary to delete the previous blank values.

The analysis is divided into four parts (cleaning, blank test, analysis and final cleaning).

Initially, it is necessary to clean the vessel, which later serves to place the sample. As with any analysis, a blank reading is performed first. This procedure is performed a total of three times. After that, the container is removed with tweezers, placed on a scale, and 10 to 50 mg of the sample is weighed. The tray is returned to the apparatus and the reading follows.

This analytical method has been verified for a range of analyte concentrations: first concentration level: 0.10 ng/g to 27 ng/g, second concentration level: 100 ng/g 400.00 ng/g. For concentrations that are above the measurement range, sample dilutions are made to concentrations within the measurement range.

After preparing samples of a known concentration, they were analyzed by the method of atomic absorption spectroscopy on an atomic absorption spectrometer, SHIMADZU AA-6650.

Statistical data processing was performed with the aim of determining the existence of a statistical difference between the samples, and with the aim of determining the level of significance if the difference exists. A one-way analysis of variance (ANOVA) was used for the analysis, in order to determine statistical significance. When a statistically significant influence of the type/origin of fish on the content of heavy metals was determined, the LSD test was applied to determine between which modalities there is a statistically significant difference. Student's test was used for statistical analysis of mercury.

Results

The highest content of lead (Pb) in freshwater fish, but also in comparison with sea water fish, had 0.0382 (mg/kg), while the lowest content was recorded in brown trout (0.0015 mg/kg). If sea water fish are observed individually, it can be seen that Gilt head sea bream had the lowest lead content of sea fish (0.0024 mg/kg), while mackerel had the highest lead content (0.0088 mg/kg). The following tables 1 and 2 shows the values of lead (Pb) content in the tested fish samples. By reviewing the average values of the content of lead in the tested samples of fresh fish, it can be concluded that all the tested samples meet the requirements prescribed by the Ordinance, given that the amount of lead in none of the tested samples is higher than the maximum allowed value of 0.3 mg/kg.

Table 1. The content of lead in marine fish

Content Pb	I control (mg/kg)	II control (mg/kg)	III control (mg/kg)	Mean value
Blue hake	0,0037	0,0017	0,0026	0,0027
Gilt head seabream	0,0035	0,0013	0,0024	0,0024
Sardines	0,0036	0,0016	0,0024	0,0025
Mackerel	0,0109	0,0067	0,0088	0,0088
Tuna	0,0044	0,0016	0,003	0,0030

Table 2. The content of lead in freshwater fish

Content Pb	I control (mg/kg)	II control (mg/kg)	III control (mg/kg)	Mean value
Rainbow trout	0,0060	0,0043	0,0051	0,0051
Brown trout	0,0004	0,0029	0,0013	0,0015
Carp	0,006	0,0016	0,004	0,0039
Chub	0,0403	0,0359	0,0383	0,0382
Gibel carp	0,0118	0,0098	0,0106	0,0107

In the research conducted by Alić et al., (20024), the lead content in trout is equal to the results obtained in this research. That is, the content of lead (Pb) in the muscle tissue of fish (mg/kg) from the Una, Vrbas and Drina basins was 0.32 mg/kg, 0.35 mg/kg, 0.57 mg/kg, with the fact that 20 .0 g of sample, while in this research 0.5-1.5 g of sample was used.

Tables 3 and 4 show that mackerel had the highest cadmium content, while carp had the lowest content. If we look only at marine fish, mackerel had the highest cadmium content, while Gilt head sea bream had the lowest cadmium content. Of the freshwater fish, the highest content had Chub, while the lowest content was carp. By reviewing the average values of the cadmium content in the tested samples of fresh fish, it can be concluded that all the tested samples meet the requirements prescribed by the Ordinance, given that the amount of cadmium in none of the tested samples is higher than the maximum allowed value of 0.05 mg/kg, i.e. 0 ,10 mg/kg for mackerel and tuna, and 0.30 mg/kg for sardine.

Table 3. The content of cadmium in marine fish

Content Cd	I control (mg/kg)	II control (mg/kg)	III control (mg/kg)	Mean value
Blue hake	0,0048	0,0048	0,0048	0,0048
Gilt head seabream	0,0002	0,0001	0,0002	0,0002
Sardines	0,0033	0,0036	0,0034	0,0034
Mackerel	0,0051	0,0055	0,0053	0,0053
Tuna	0,0026	0,0027	0,0027	0,0027

Table 4. The content of cadmium in freshwater fish

Content Cd	I control (mg/kg)	II control (mg/kg)	III control (mg/kg)	Mean value
Rainbow trout	0,0002	0,0001	0,0001	0,00013
Brown trout	0,0000	0,0001	0,0001	0,00006
Carp	0,0000	0,0001	0,0000	0,00004
Chub	0,0009	0,0009	0,0009	0,00091
Gibel carp	0,0003	0,0005	0,0004	0,00038

Sardine had the highest arsenic content (1,1668 mg/kg), while carp had the lowest content (0,008519 mg/kg). If we look only at marine fish, sardines had the highest arsenic content (1,1668 mg/kg), while tuna had the lowest arsenic content (0,0197 mg/kg). Of the freshwater fish, brown trout had the highest content (0,066375 mg/kg), while carp had the lowest content (0,008519 mg/kg).

Table 5. The content of Arsenic in marine fish

Content Cd	I control (mg/kg)	II control (mg/kg)	III control (mg/kg)	Mean value
Blue hake	0,4998	0,4985	0,4992	0,4992
Gilt head seabream	0,1177	0,1218	0,1198	0,1198
Sardines	1,1991	1,1345	1,1668	1,1668
Mackerel	0,3032	0,2885	0,2958	0,2958
Tuna	0,0176	0,0220	0,0195	0,0197

Table 6. The content of Arsenic in freshwater fish

Content Cd	I control (mg/kg)	II control (mg/kg)	III control (mg/kg)	Mean value
Rainbow trout	0,0095	0,0210	0,0156	0,015352
Brown trout	0,0589	0,0740	0,0662	0,066375
Carp	0,0052	0,0118	0,0085	0,008519
Chub	0,0253	0,0405	0,0329	0,032893
Gibel carp	0,0200	0,0574	0,0387	0,038681

Rulebook, given that the amount of arsenic in none of the tested samples is higher than the maximum allowed value of 2.0 mg/kg, i.e. 4.0 mg/kg for white sea fish, and 8.0 mg/kg for tuna. Therefore, all tested samples can be used in food without fear of arsenic poisoning.

Table 7. The content of Mercury in all samples

Content Hg	**Measured value (mg/kg)**
Blue hake	0,09919
Gilt head seabream	0,06033
Sardines	0,05009
Mackerel	0,07886
Tuna	0,09221
Rainbow trout	0,01509
Brown trout	0,02717
Carp	0,09881
Chub	0,00334
Gibel carp	0,08776

By reviewing the mercury content values obtained in the tested samples of fresh fish, it can be concluded that all the tested samples meet the requirements prescribed by the Ordinance, given that the amount of mercury in none of the tested samples is higher than the maximum allowed value of 0.5 mg/kg for muscle meat fish, i.e. 1.0 mg/kg for tuna.

Conclusion

The obtained results, on the basis of which conclusions can be drawn, showed that the lead(Pb) content in the tested samples of fresh fish ranged from 0.0015 to 0.0381 mg/kg, which is within the allowed values prescribed by the Rulebook.The content of cadmium (Cd), arsenic (As) and mercury (Hg) was within the limits allowed by the regulations.Since the statistical analysis of the experimental data showed the existence of statistically significant differences in the content of heavy metals depending on the habitat of the fish, it can be concluded that the habitat of the fish affects the content of heavy metals in the fish. Therefore, in addition to differences between marine and freshwater fish in the content of heavy metals, it has been noted that there are also differences within "one" habitat. This may be due to the proximity of roads, industry, etc.

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BIOACTIVE POWER OF LYOPHILIZED PLANT RAW MATERIALS: EVALUATION AND ASSESSMENT OF SUITABILITY FOR THE PRODUCTION OF NATURAL FOOD SUPPLEMENTS AND ANTIMICROBIAL DRUGS

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Abstract: Superior preservation and utilization of bioactive compounds (BAC) from plants, secondary or by-products of fruit and vegetable processing, can be achieved by lyophilization, witout chemicals used, so it is possible to deliver the highest level of BAC to consumers or specific groups, with environmentally friendly and effective products or dietary supplements. In this review paper, we will present the experiences of researchers worldwide and the opinion of our team on the possibilities of creating foods or dietary supplements modified by BAC from various synergistic phytocomplexes, superior bioactivity: in the lowest concentrations, proven positive effects on human health, with intact sensory and techno-functional properties of enriched food, attractive to consumers.

Keywords: lyophilisates, bioactivity, plant raw material, food supplements, antimicrobials

1.1. **Introduction**

In order to preserve and utilize BAC from bio-materials (plants, secondary or by-products of fruit and vegetable processing), certain extraction green method (GEM) and lyophilization will be applied. Obtained products (plant extracts - PE and lyophilizates - LYO) might be applied directly into the food matrix or for the production of various supplements or antimicrobial (AM)

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drugs. Vast of BAC are thermally unstable. Optimization of extraction procedure plays an important role in their preservation.

After determination of chemical composition and sterility control, LYO can be used in processing foods of plant/animal origin, heading towards the highest possible beneficial health effects, but in the lowest concentration in order to preserve sensory properties of foods. In October 2015, WHO through its International Agency for Research on Cancer (IARC) published a monograph classifying meat products as carcinogenic (Group I), and red meat as a potential carcinogen (Group IIa), based on the published results of human and animal research on the connection between meat consumption and the occurrence of colorectal and other types of cancer. Meat is a healthy food item and has quality proteins (high biological value) and high content of essential minerals and B-vitamins, but it is poor in antioxidants. The addition of phytocomplexses of LYO with antioxidant (AOX) effects to processed meat leads in healthier/functional products. Innovative approaches in preservation are needed for both conventional and organic meat products to improve sustainability and reduce potentially harmful effects of processing and meat consumption. Phytocomplexses with high contents of specific phytochemicals obtained from varied plant materials and by-products from the processing of these materials could have synergistic AM effects, prevent the growth of pathogens and bacteria that are the cause of failure in meat products, and improve their quality and safety. In fermented dry sausages, potentially toxic nitrites were replaced with PE of Serbian endemic plant (Kurćubić et al., 2014).

In Serbia and ex-Yugoslavia (due to the abundance of healthy natural resources and the development of the processing industry - intensive development of wineries and distilleries), we consider the extremely important fact, in order to promote the development of the circular economy and environmental protection: fruit processing by-products are rich in BAC, dietary fibre and unsaturated fatty acids, such as by-products of grape processing, grape pomace (GP), which contained a variety of phenolic compounds which include phenolic acids, phenolic alcohol, flavan-3-ols and flavonoids (Yu et al., 2013). Our opinion and attitude are that also important to know and apply LYO rich in BAC, used in folk and traditional medicine (weeds, medicinal plants and herbs): Wild Garlic - Ramson (*Allium ursinum* L.); Different types of buckwheat (Common buckwheat: *Fagopyrum esculentum* and Tartary buckwheat: *Fagopyrum tartaricum*); Danewort (*Sambucus ebulus*), Nettles (*Urtica dioica*). In the subsection 1.3. we will explain our opinion and support it with data from the literature.

Butkevičiūtė et al. (2021) reported that LYO of various fruits (plums, apples, peaches, pears), berries (blueberries, cranberries, strawberries), and vegetables (carrots, beets) can be applyed in the food industry for enriching and fortification of different types of meals, yogurts, ice cream, cocktails, cereals, drinks, juices. LYO fruit powder can also be added like food supplements thus increasing the content of BAC (Delpino-Rius et al., 2015; Ruszkowska et al., 2019; Bajić et al., 2020). Freeze-drying, unlike other preservation methods, effectively protects the thermolabile BAC stored in the fruit. No chemical substances are used during the freeze-drying process, which enables the delivery of the highest level of BAC to consumers through the supply of safe, environmentally friendly and effective products or dietary supplements (Butkevičiūtė et al., 2021).

The purpose of predicted goals is that food modified with BAC from a various phytocomplexes which will achieve synergy and maximize effects: strongest effects in the lowest concentrations, proven positive impacts on human health, with intact sensorial and techno-functional properties of fortified foods appealing to consumers.

1.2. ***Lyophilization*** (***freeze-drying***)

The lyophilization procedure (freeze-drying) represents the most efficient way of drying different materials by sublimation (a physical phenomenon) - a direct transition between the solid and gaseous state without passing through the liquid phase, so that the frozen product is dried under vacuum, without thawing. This procedure allows (plant) materials to be preserved without damaging the BAC in them, with minimal changes in color, smell and taste (Kittibunchakul et al., 2023).

It is speculated that lyophilization is a superior non-convective drying technique compared to other preservation techniques (Gaidhani et al., 2015). Properly preserves BAC present in plants and spices. Several studies have been carried out to prove this position, primarily that lyophilization properly protects the quality and quantity of BAC and volatile substances originating from raw plant material (Abascal et al., 2005; Özcan et al., 2005; Kittibunchakul et al., 2023). Due to the low preservation (operating) temperature and a shorter processing time, several research findings indicate lyophilization as the optimal drying method to preserve the fresh aroma of spices (Antal, 2010). Lyophilized and hot-air dryed at 50 °C were promising approachs to obtain fruits revealed higher retention of predominant BAC, especially for AOX substances

(Butkevičiūtė et al., 2021). There are also studies in which the results revealed certain negative effects of freeze drying (Chang et al., 2006).

Salamon (2021) reports that it is important to find optimal methods and procedures for their preservation for BACs that have been proven to prevent the progression of viruses and their spread from the host cell, so that the preparations created from them could be patented and applied in different forms and different ways of drug application (administration).

1.3. *Various applications of lyophilisate in the preparation of healthier and functional food*

The purpose of predicted goals is that food modified with BAC from a phytocomplexes which will achieve synergy and maximize effects: strongest effects in the lowest concentrations, proven positive impacts on human health, with intact sensorial and techno-functional properties of fortified foods appealing to consumers. The global market for dietary supplements is growing exponentially (9.1% annualy) and is expected to reach $327.4 billion by 2030 (Grand View Research, Inc., 2023). Consumers are becoming more aware and interested in health and wellness issues, so in an effort to improve their overall well-being, they are choosing nutritional supplements as a convenient and affordable means to meet their nutritional needs.

Grape pomace (GP) has the potential to serve as a functional food ingredient. Bread and pasta could be enriched with up to 5-20% dried and milled GP flour - AOX potential of bread increases (Tolve et al., 2021). The new snack, bar, or porridge, which includes GP, supported with buckwheat and other grains, could be used in a formulation of new healthier foods (Santiago-Ramos et al., 2022). The addition of GP powder significantly improved the content of free phenolics, highly bioavailable, that are scarce in grains, and thus the nutritional value of biscuits (Nakov et al., 2022) and storability in terms of higher shelf life. Chocolate and chocolate spreads can be enriched with many different BACs using minimally processed GP. The AOX activity of aqueous extracts of GP increases after intestinal digestion. These inhibited the growth of the tested pathogens, maintained shelf life, and improved the safety of ready-to-eat food products (Caponio et al., 2022).

Dried GP could be used as a supplement in chocolate spread formulations at a maximum of 10% without a bitterness effect (Acan et al., 2021).

More recently, Baj et al. (2016) developed a cream formulation with potential AOX properties, with a LYO of *Ligusticum mutellina* as a source of

BAC. The recipe for the composition of the cream provided a good profile of the release of polyphenolic compounds, which indicates that the examined PE (due to the retained high content of phenolic acids) can play a key role in the creation and production of AOX creams.

Kurćubić et al. (2023a) examined the chemical properties of freshly squeezed wild garlic extract (FSWGE) in burgers (BU). Technological and sensory properties of such fortified burgers (BU) were determined. LC-MS/MS analyses identified thirty-eight volatile BAC. Allicin prevalence (11.375 mg/mL) is the key parameter determining the amount of FSWGE added in raw BU (PS-I 1.32 mL/kg, PS-II 4.40 mL/kg, and PS-III 8.79 mL/kg). MIC and MBC values of the FSWGE and evaporated FSWGE (EWGE) were determined against the 6 microorganisms. FSWGE usage resulted in a reduced risk of *Listeria monocytogenes* (MIC = MBC = 90 mg/mL), *Escherichia coli* and *Staphylococcus aureus* (MIC = 90 mg/mL; MBC ⩾ 100 mg/mL), and *Salmonella enteritidis* and *Enterococcus faecium* (MIC = 100 mg/mL; MBC > 100 mg/mL) in BU. Reformulation of BU with FSWGE did not negatively affect the technological and physico-chemical properties during both cold and freeze storage. Modified BU received mostly higher sensory scores compared to control (color, texture, taste and general acceptability were evaluated with higher grades), revealed the great potential of FSWGE usage in the creation of safe meat products with prolonged shelf life. Addition of FSWGE reduced weight loss after grilling, without alter the proximate composition.

Buckwheat, common (*Fagopyrum esculentum*) and Tartary buckwheat (*Fagopyrum tartaricum*) has attracted research interest due to high efficiency as a functional food. Various Tartary buckwheat food products, such as alcoholic beverages, vinegar, tea, noodles, porridge, biscuits, cakes, and sprouts, become commercially available in China (Zhu, 2016). Both species have excellent quantity of phenolic compounds, rutin and quercetin. Rutin, quercetin, and kaempferol could eliminate the DPPH radical effectively (Zhong et al., 2022).

Danewort (Dwarf Elder, *Sambucus ebulus*) fruit has multiple uses and its roles as an immunological stimulator, anti-inflammatory processes, antioxidative and anticarcinogenic. Danewort is rich in health-promoting phytochemical such as polyphenols and anthocyanins, with significant AOX activity. Comparing with other *Sambucus* species, danewort contain the highest levels of beneficial phenolics (Senica et al., 2019).

The lyophilized nettle PE (in the amount of 2%) enriched the chocolate in total polyphenolic content, chlorogenic acid, and flavonoid derivatives showed more intense bitterness and astringency (Belščak-Cvitanović et al., 2015).

1.4. ***Antimicrobial (AM) activity of lyophilized or GEM obtained plants - preservation of public health***

In the following section, we will present findings on the AM activity of lyophilized plants.

Antimicrobial (AM) resistance is considered a significant threat to the public health systems not just in developing countries but throughout the world (Prestinaci et al., 2015; Founou et al., 2017). Due to occurrence of multidrug-resistant (MDR) bacterial strains, attention is now being shifted towards BACs isolated from plant species communing used as herbal medicine, as they may produce a new potent source of antibacterial and antifungal activities (Maiyo et al., 2010; Erfan and Marouf, 2019). The AM properties of plants related to their ability to give several secondary metabolites of relatively complex structures possessing AM activities (Souza et al., 2005; Matasyoh et al., 2009). At the moment, the demand for medicinal plants for the isolation of natural BAC has increased rapidly (Cameron et al., 2005; Rai and Carpinella, 2006).

Kurćubić et al. (2023b) were screening the AM activity of an ethanolic extract of *Kitaibelia vitifolia* against 30 MDR bacterial strains isolated from healthcare-associated infections. Minimum inhibitory concentrations (MICs) of the samples against the tested bacteria were determined using the microdilution method. MDR bacterial strains were characterized using standard biochemical tests and the commercial identification systems API 20 NE and API 20 E. The highest level of sensitivity to *K. vitifolia* PE was confirmed in 88.89% of *Klebsiella* spp. isolates, *E. coli* ATCC 25922, two strains of MRSA (1726, 1063), *Acinetobacter* spp. strain 1578, and VRE strain 30, like *Enterococcus faecalis* ATCC 29212 (MIC =< 2.44 μg/mL). The lowest sensitivity was exhibited by 75.00% of *Acinetobacter* spp. (strains 1577 and 6401), where the highest values for MICs were noted (1250 μg/mL). *K. vitifolia* PE could be a potential basis for designing and creating a new, superior, and natural AM drugs to combat infections caused by MDR strains of bacteria originating from hospital or public environments.

The AM activity assays of the methanol extract of Tartary buckwheat sprouts showed excellent activity toward most of the tested fungi and bacteria. Quercetin presents the main active component for contributing to AM activity. Tartary buckwheat exhibits anticancer, anti-hypertension, antidiabetic, cholesterol-lowering, and cognition-improving activities (Zhu, 2016). Doctors-clinicians call buckwheat a "smart bioactive supplement", it has proven positive effects when (according to precise recommendations) it forms the basis of meals for oncology patients.

Cvetkovic et al. (2023) investigated the AM activity of lyophilized fruit juice (RPJL—Redpoll juice lyophilizate) and waste (RPWL—Redpoll waste lyophilizate) obtained from red currant (*Ribes rubrum* L.) variety Redpoll on different G+ and G– bacteria and yeast (*Candida albicans*). Several studies have found that they have various biological effects, such as antiproliferative, anticancer, AM, antiinflammatory, antidiabetic, and antioxidant (Tsuda et al., 2003; Aneta et al., 2013; Folmer et al., 2014; Dey et al., 2016). Berries rich in phenolics also exhibit AM action against pathogenic bacteria (Heinonen, 2007). Tested RPJL and RPWL showed moderate AM activity. Cvetkovic et al. (2023) revealed that red currant RPJL did not inhibit G– bacteria (*E. coli*, *P. aeruginosa*, *S. enteritidis*, *P. mirabilis*, and *E. aerogenes*), while was effective on all G+ bacteria (*B. cereus*, *S. aureus*, *E. faecalis*), except *L. monocytogenes*. Red currant RPWL inhibited activity of both G+ and G– bacteria, except *E. coli*. RPWL shown stronger AM effects compared to RPJL, especially on G– bacteria. The significance of the bacteriostatic activity of RPJL and RPWL against *B. cereus* is that they can stop this causative agent of alimentary infections accompanied by nausea and vomiting (Kotiranta et al., 2000). AM power of RPWL against *L. monocytogenes* may prevent listeriosis, which causes food contamination (Ramaswamy et al., 2007). RPJL and RPWL might be used as a potential preservative in the food industry (Cvetković et al., 2023).

Fireweed (*Chamaenerion angustifolium* L.) is a well-known medicinal plant due to its anti-inflammatory, AOX, antibacterial, analgesic, and anti-cancer properties (Kuznetsova et al., 2020; Sõukand et al., 2020). Fireweed herb contains about 1–2% of flavonoids (aglycones myricetin, kaempferol, 8-methoxykaempferol, quercetin, and their glycosides (Hevesi Tóth et al., 2009; Karasiewicz et al., 2011). Gram-positive flora (G+: *Staphylococcus aureus*, *Bacillus cereus*) as evidenced by the bactericidal effect was the most sensitive to the native L.

The neuraminidase enzyme is found on the surface of the virus and allows it to leave the host cell. Neuraminidase is blocked by natural substances (e.g. flavonoid anthocyanin - cyanidin-3 sambubiocid, in the amount of 8200 ng.ml-1, isolated from the fruits of *Sambucus nigra* L.) (Swaminathan et al., 2013; Wu et al., 2015). An antiviral preparation can be a LYO of anthocyanins, isolated from elderberry fruits. Anthocyanins are unstable under different conditions, and lyophilization stabilizes them for a long time by removing water or other components from the solution, which are thermolabile and sensitive to pH, light or structural changes. Final anthocyanin product has the potential to be used as a food supplement, gelatin tablets, but also to be administered by injection. The berries contain a coloring agent that can be added to confectionary spreads and creams, sweets, dough, jams, and marmalades, as well as in liqueurs and wine products to give color (Burak, 2020). The listed LYO are useful for the prevention or therapy of cardiovascular and neurodegenerative diseases, eye, muscle and sarcopenia disorders, even for the possible dietary treatment of Duchenne muscular dystrophy (Wicks et al., 2018). Several scientific experiments (ZakayRones et al., 1995; Wilks et al., 2012) have shown that natural components (anthocyanin flavonoids) extracted from the fruits of the elderberry (*Sambucus nigra* L.) prevent the spread of swine flu infection by preventing its entry into the H1N1 virus from entering the lung host cell. *In vitro* experiments have shown that these flavonoids are able to prevent the entry of various parasitic strains of influenza viruses into human cells (Ishiguro et al., 2018). Anthocyanin flavonoids extracted from the fruits of elderberry have ability to increase the production of interferons (IFN-β) (Barak et al., 2001; Frokiaer et al., 2021).

According to the scientific literature, *Philadelphus coronarius* possesses AM properties. It has been previously studied by the microdilution method for G+ and G– bacteria as well. It was confirmed that the extract had a strong antibacterial effect (Nagy et al., 2000). In experiments of Peto et al. (2022), the antibacterial properties of the lyophilized flower and leaf were studied, followed by antifungal investigations. First, microdilution was carried out. In this investigation, the *P. coronarius* flower reduced the viability of *E. coli* and the leaf decreased the cell viability of *S. aureus* and *E. coli*. AM testing was continued with the *in vitro* time-kill test, whereby the effect of *P. coronarius* was studied on the growth of microbes over time. The growth of *S. aureus* and *C. albicans* was delayed by the leaf, while the flower did not elicit any changes. However, these results are not consistent with the results of the microdilution, since it is obvious that the leaf has AM potential. The time-kill test is considered to be the more accurate of the two methods, because it includes time as an important factor in the experiment. Previous chemical studies have revealed the flavonoid, triterpene, coumarin and phenolic-acid content of the herb (Klecáková et al., 2004). *P. coronarius* is said to exhibit antibacterial effects, which is confirmed by the experience of folk medicine using it in the treatment of different gynecological illnesses (Egészségtér, 2022).

Dinslage et al. (2002) reported about the greater intraocular bioavailability of fluorescein from an innovative water free, lyophilized drug delivery system for use in ophthalmology compared to conventional fluorescein eye drops (preservative-free), and implies that such an approach may be useful for supplying the eye with BAC. It was confirmed by the existence of findings that several plant species can be used for treatment of the ophthalmological problems (Calvo and Cavero, 2016; Alghamdi et al., 2023). Salazar-Gómez et al. (2023) systematized and published literature data on medicinal plants in the prevention and treatment of eye diseases in Mexico.

Conclusion

This new concept of implementation and processing of plant material can lead to new ideas for its application to other plant species or by-products of plant processing (weeds, herbs, fruits, vegetables, cereals, etc.). It also offers the possibility of cooperation between researchers of different scientific profiles: technologists, microbiologists, veterinarians, IT experts, and chemists. Results achieved through this approach may become a ground base for future projects in different research institutions and teams concerning the transformation of plant raw materials into final products (food) with strong bioactive potentials.

A major practical benefit of the applying of LYO will be new recipes for fortified/enriched food and modeling the most appropriate GEM and a combination of animal and plant materials for a specific food. The similar projects may attract also other end-users such as interested institutions.

A significant outcome of the innovative technology will be a clean label product of animal and plant origin, with potentially positive effects on human health, which could be incorporated into industrial processes.

The results of the innovative technology transfer will also contribute to improve awareness of new ideas and concepts for further experiments/projects.

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EXAMINATION OF THE SURVIVAL OF *Lactobacillus plantarum* AND *Lactobacillus rhamnosus* DURING KOMBUCHA FERMENTATION

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Abstract: Kombucha is a traditional fermented beverage, which is usually prepared by fermenting sweetened black tea using a symbiotic consortium of acetic acid bacteria and yeasts. Kombucha exhibits several functional properties, it still can't be labelled as a probiotic product. In this study, *Lactobacillus plantarum* and *Lactobacillus rhamnosus* were added separately at the start of the fermentation to examine their survival during fermentation conditions. Microbiological and chemical parameters were tested during 5 days of Kombucha fermentation. Sample inoculated with *L. plantarum* demonstrated better survivability, but number was not enough to label the beverage as probiotic.

Keywords: kombucha, lactic acid bacteria, fermentation, probiotics

Introduction

Kombucha is a traditional fermented beverage that originated in northeastern China (Manchuria region) and is believed to have been used since 220 BC. With the expansion of trade routes, this beverage reached Russia and Eastern Europe, and later appeared in Germany in 20th century. Nowadays, Kombucha gained popularity mainly because of its claimed therapeutic properties such as antioxidant, anticancer, antidiabetic, antimicrobial, detoxifying, immune-stimulating effects etc, and can be purchased in retail stores across the world (Coelho et al., 2020).

Kombucha is usually prepared by fermenting sweetened black tea. The fermentation is carried out by a symbiotic consortium of acetic acid bacteria (AAB; most dominantly *Gluconacetobacter xylinum*) and yeasts (species of the genera *Saccharomyces*, *Torulopsis*, *Pichia*, *Brettanomyces*, *Zygosaccharomyces*, *Candida* and *Saccharomycoides*) embedded in a cellulose film, that is typically called SCOBY (Symbiotic Consortium of Bacteria and Yeast) or "tea fungus" (Coelho et al., 2020).

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The role of yeasts during fermentation is to hydrolyze sucrose from the medium to glucose and fructose and produce ethanol via glycolysis. On the other hand, AAB use glucose to produce gluconic acid and ethanol to produce acetic acid (Coelho et al., 2020). Some studies also reported the presence of lactic acid bacteria (LAB) in Kombucha broth as well as in the cellulose pellicle (Sengun and Kirmizigul, 2020). In the study conducted by Marsh et al. (2014), *Lactobacillus* and *Lactococcus* were the most dominant species in SCOBY. LAB present a very important heterogeneous group of phylogenetically related microorganisms that mainly produce lactic acid as a by-product during metabolic activities (Mozzi, 2016). In some studies, Kombucha is presented as a probiotic beverage, since it contains live microorganisms, but the presence and number of LAB is often inconsistent and there is still no evidence of its probiotic potential (Vargas et al., 2021). The aim of this study was to investigate the viability of *L. rhamnosus* and *L. plantarum* strains during Kombucha fermentation to examine the possibility of producing a beverage that meets the requirements of a probiotic product.

Materials and methods

The tea fungus used in this study and its cultivation was the same as described in the previous paper by Cvetković et al. (2019). After preparation of inoculated media, three series of jars were prepared. First one was the control series (Control Kombucha) which was not inoculated with LAB. Second series was inoculated with a suspension of *L. plantarum*, at the starting day (Start L.pl.) of fermentation. Third series was inoculated with *L. rhamnosus* also at the beginning (Start L.rh).

Lactobacillus strains included in this study were *L. plantarum* ATCC 14917 and *L. rhamnosus* ATCC 53103. Preparation of suspensions was done according to Cvetković et al. (2019).

Sampling of fermentation broth during tea fungus cultivation was performed every day for further analysis. Each glass jar was sampled only once to avoid potential contamination. Methods of analysis were: pH values, titratable acidity (TA), number of yeasts, AAB and LAB and described in paper by Cvetković et al. (2019). Each quantity was measured three times and results expressed as mean ± standard deviation.

Results and discussion

The pH value of sweetend uninoculated black tea was 7.28 and decreased to 4.16 after inoculation. As shown in Figure 1, pH values decreased during first two days for about 1 unit, and after ter that, pH continued to further decrease a bit slower during the remaining 3 days. pH values of all fermentations reached between 2.78 and 3.06 on 5th day of fermentation. This typical trend is in correlation with other studies (Jayabalan et al., 2008; Cvetković et al., 2019).

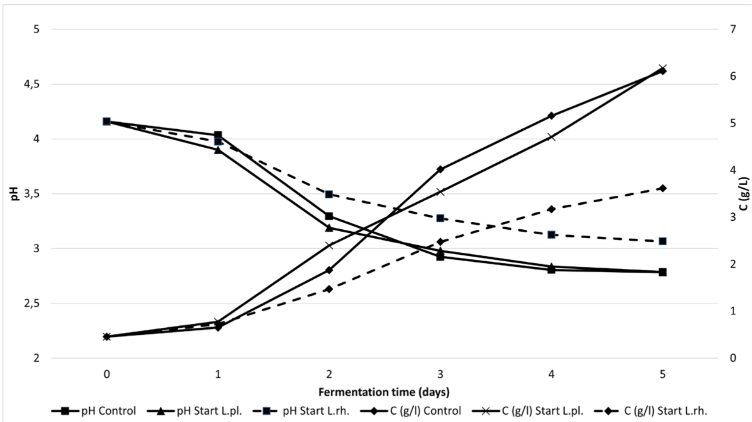

Figure 1. The changes of pH and titratable acidity (TA) (g/L) during Kombucha fermentations.

TA of cultivation medium before inoculation was 5.86 g/L and decreased to 0.46 g/L after inoculation (Figure 1). Kombucha consumers confirmed that in order to obtain a pleasantly sour, sweetened beverage, fermentation should be terminated when TA fermentation broth reaches 4–4.5 g acetic acid/L. TA gradually increased and reached the optimal values between 3 and 4 days for both Control and sample inoculated with *L. plantarum*. A sligltly slower increase was noticed in the sample inoculated with *L. rhamnosus* where TA reached 3.6 g/L by the end of fermentation.

The number of yeasts after inoculation was 5.15 log cfu/mL and increased for about 1-1.5 unit in all of the samples after 24 h (Figure 2). During the fermentation of all of the samples, number of yeasts gradually increased and reached its maximum on the third day of fermentation after which it started to lower. A similar result was obtained by Majid et al. (2023) where the maximum number of yeasts was noticed on fourth day, after which a slow decrease in numbers appeared. The number of AAB after inoculation was 5.26 log cfu/mL and increased after 24 h in all of the samples. Number of AAB differed in all of the samples and reached maximum values on day 2 for Control and day 3 for Start L.rh., after which a slow decline was noticed. Yang et al. (2008) concluded that the addition of LAB during fermentation could support the growth and reproduction of other present bacteria, including AAB, which could explain the unusual growth of AAB during the 5th day of fermentation in Start L.pl. sample. Also, the higher number of AAB in this sample makes sense in comparison to acetic acid production, since TA values were also higher than ones detected in sample inoculated with *L. rhamnosus*.

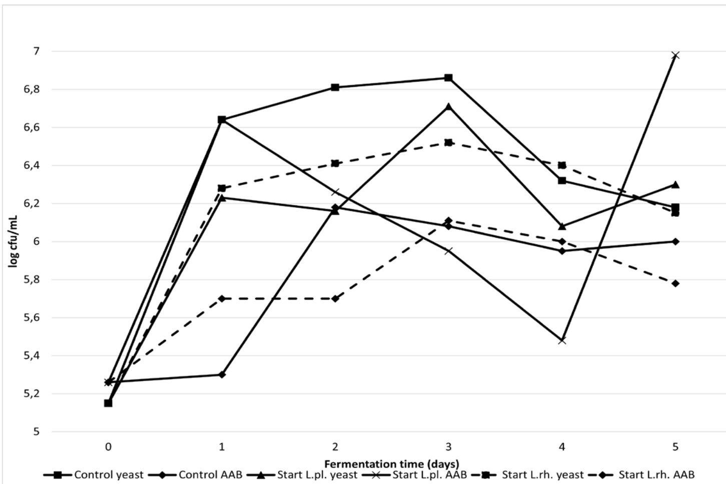

Figure 2. The changes of number of yeast and AAB (log cfu/mL) during Kombucha fermentations.

The presence of LAB in Control Kombucha sample was not detected (Table 1). The number of LAB was determined 2 h after the addition at the beginning of fermentation. As seen in Table 1, the number of *L. plantarum* decreased from 6,81 log cfu/mL at the beginning, by 0.3 – 0.5 units until the second day, after which it continued to rapidly decrease reaching a value of 4.3 log cfu/mL on day 3. The number after 3rd day was less than 100 cfu/mL. As for *L. rhamnosus*, the number decreased in the first 24 h for about 2 units and reached a minimum value on the second day. Viability of *Lactobacillus* spp. strains can depend on a lot of factors such as fermentation medium composition, pH, and temperature conditions. The optimum temperature for LAB growth can vary from 25 to 40 °C, so the fermentation temperature of 28 °C was determined not to be a limiting factor. On the other hand, optimal pH values for LAB growth are between 4.5 and 6.5, for some strains even lower (Śliżewska and Chlebicz-Wójcik, 2020). The requirement for probiotic products is that they must have minimum amount of viable cells that vary from 10^6 to 10^8 cfu/g (mL) (Majid et al., 2023), so Kombucha beverage in this study could not be labeled as a probiotic product. New terms of paraprobiotics and postbiotics appeared recently and indicate that even though no LAB remained viable after 3 days, it is not excluded that the inactivated and lysed cells don't still exhibit functional properties. It has been determined that some health benefits of physiologically active bacteria are not strictly related to their viability and that their effectiveness is based on microbial metabolites or complex molecules still contained in inactivated cells (Siciliano et al., 2021).

Table 1. Number of *L. plantarum* and *L. rhamnosus* (log cfu/mL) during Kombucha fermentation.

Sample	Fermentation time (days)					
	0	1	2	3	4	5
Control	nd	nd	nd	nd	nd	nd
Start L.pl.	6.81 ± 0.03	6.41 ± 0.1	6.02 ± 0.05	4.39 ± 0.09	< 2	< 2
Start L.rh.	6.1 ± 0.1	3.84 ± 0.06	2.84 ± 0.06	< 2	< 2	< 2

*nd - non detected

Conclusion

The addition of LAB at the beginning of the fermentation did not influence the standard pH changes, but it did affect the duration of fermentation and microbiological parameters. By adding LAB strains, their number drastically decreased during the 5 days, most probably because of unfavorable medium conditions, so Kombucha in this study could not be used as a probiotic beverage. To enable the survival of LAB in Kombucha, further research could include addition of LAB during the fermentation when conditions in the medium are more favorable or addition to the finished product after the fermentation, thus monitoring the viability during storage.

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ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF *ZIZYPHUS JUJUBA* L. LEAF EXTRACTS

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Abstract: The aim of the present study was to investigate the biological activities of the extracts obtained from leaf of *Ziziphus jujuba*. To reach this purpose, total phenolic content (TPC), total flavonoid content (TFC), DPPH and ABTS radical scavenging activity, as well as antibacterial activity extracts were evaluated. The content of total phenols in leaf ranged from 22.71 to 28.69 mg GAE/g, the total content of flavonoids ranged from 5.15 mg RE/g to 8.25 mg RE/g depending on the applied extraction method. All extracts showed very high antioxidant activity. The ethanol extracts of *Ziziphus jujuba* leaf were screened for antibacterial activities against *Salmonella typhimurium, Salmonella enteritidis, Listeria ivanovii, Listeria monocytogenes, Staphylococcus aureus* and *Pseudomonas aerogenes* using microdilution method. Furthermore, the highest antibacterial activity was observed against *Salmonella typhimurium* and *Salmonella enteritidis*. The present results suggested the promising antioxidant and antibacterial properties of *Ziziphus jujuba* leaf extracts, which can be used in the fabrication of functional bioactive ingredients for different purposes.

Keywords: phytochemicals, antibacterial evaluation, *Ziziphus jujuba*

Introduction

Chinese jujube (*Zizyphus jujuba* Miller) with over 700 subspecies has been used during thousands of years of cultivation in the temperate and subtropical areas of the Northern Hemisphere (Wang et al., 2016) and as a crude drug in traditional medicine for the purpose of analeptic, palliative, antibechic actions (Li et al., 2007).

Different parts of jujube are rich in medicinal properties such as analgesic, antiseptic, and antidiabetic. Jujube fruit is rich in vitamins A, B, and C, as well

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as minerals such as calcium, phosphorus, and iron, it contains protein, fat, carbohydrates and various compounds such as alkaloids, flavonoids, tannins, saponins, sterols and fatty acids (Ozturk et al., 2021). Jujube also has significant antioxidant properties that can neutralize the activity of free radicals, so its fruit is involved in traditional medicine (Tatari et al., 2016). This antioxidant activity has been attributed to the high level of phenolic compounds.

The jujube leaf, which is the main byproduct of jujube, has also been used for thousands of years (i.e., to improve sleep, to nourish the heart and soothe the nerves, and to reduce hemorrhaging and diarrhea). Jujube leaves are rich in bioactive components and have various physiological and pharmacological functions (the aqueous ethanol extracts of the jujube leaf were used as energetic constituent for hepatosis and wound healing in animal trials) (Zhang et al., 2019). In addition, natural antioxidants have the capacity to improve food quality and stability, and can also act as nutraceuticals to terminate free radical chain reactions in biological systems, and thus may provide additional health benefits to consumers (Sampath Kumara et al., 2012). Phenolic and flavonoids were believed to be the major bioactive components in jujube leaves, which have been shown to be responsible for cardioprotective, anticancer, antidiabetic, anti-aging and neuroprotective effects.

Most studies focused on the bioactivity and chemical constituents of jujube seeds and fruits, and less attention was devoted to the jujube leaf.

The number of bioactive compounds recovered from plant materials depends on the extraction methods used. Therefore, the objective of this study is to explore the phenolic composition and antioxidants of the ethanol–water and water extracts from *Chinese jujube*, to provide sufficient experimental evidence for antioxidant activity and potential for further development and utilization of jujube.

Materials and methods

The extraction process

5 g of leaf sample (ground) was extracted with 50 mL of solvent (50% ethanol or water). The extraction methods are given below.

1. Infusion - water 80 ℃;
2. Infusion - water 100 ℃;
3. Maceration water - 22 h;
4. Maceration 50% ethanol - 22 h;
5. Ultrasonic extraction with water, 30 min.;
6. Ultrasonic extraction with 50% ethanol, 30 min.

The samples were filtered and total phenols, flavonoids and antioxidant activity were determined from the filtrate. UV/Vis spectrophotometry was used to determine the concentration of total phenols, flavonoids and antioxidant capacity. Spectrophotometric measurements were performed on a UV-VIS spectrophotometer (Cary Series 300 Agilent Technologies).

For determining antibacterial activity of leaf extract - the extracts were obtained by maceration (24h) with 50% ethanol. After decanting, the extracts were evaporated in a rotary vacuum evaporator at 45℃ to a gelatinous mass. The concentrated extract was kept in a screw cap bottle at 4℃. The extracts were dissolved in 10% dimethylsulfoxide at an initial concentration of 200 mg/mL.

Determination of total phenolic acids (TPC)

The TP contents were determined by the Folin–Ciocalteau method (Singleton et al., 1965). The extract samples (40 µL) were mixed with 3.16 water, after that 200 µL Folin–Ciocalteu was added. After reaction for 8 min, 600 µL of sodium carbonate was added. The absorbance of reaction was measured at 760 nm after 2 h of incubation at room temperature. The TP concentration was expressed as the equivalent to milligrams of gallic acid per g of dried weight (mg GAE/g DW).

Determination of total flavonoids (TFC)

The total flavonoid content was measured by a colorimetric assay. The extracts (1 mL) were added to a test tube containing 4 mL of water. Sodium nitrite solution (5%, 0.3 mL) was added to the mixture and reacted for 5 min, followed by the addition of 0.3 mL of 10% aluminum chloride. After 5 min, 2 mL of 1 M sodium hydroxide was added and supplemented with 2.4 mL of distilled water and mixed well. The absorbance of mixture was measured at 510 nm. The results were expressed as the equivalent to milligrams of rutin per g of dried weight (mgRE/ g DW).

Antioxidant capacity

Antioxidant properties were determined by the ABTS assay (Re et al., 1999). The ABTS stock solution in distilled water was prepared from 7 mM ABTS and 2.45 mM potassium persulphate, and then incubated in the dark for 12 - 16 h at room temperature. The ABTS working solution was prepared by diluting the stock solution with ethanol to an absorbance of 0.70 ± 0.05 at 734 nm. A 10 µL of extracts was mixed with 1 mL of prepared ABTS solution and mixed for 6 min. Absorbance was measured at 734 nm, with distilled water as a reference. Results were expressed as percentage of inhibition of the ABTS radical.

The antioxidant capacity of the extracts was also studied through the evaluation of the free radical-scavenging effect on the 1.1-diphenyl-2-picrylhydrazyl (DPPH) radical. An aliquot 0.1 mL of extract was mixed with 3.9 mL DPPH solution ($6 \cdot 10^5$M). The mixture was thoroughly vortex-mixed and kept in the dark for 30 min. After that, the absorbance was measured at 515 nm, with distilled water as a reference. Results were expressed as percentage of inhibition of the DPPH radical.

Antibacterial activity - minimum inhibitory concentrations

The antibacterial (AB) activity was tested against the *Listeria ivanovii, Listeria monocytogenes, Salmonella typhimurium, Staphylococcus aureus, Pseudomonas aerogenes, Salmonellla enteritidis*. Bacterial colonies from the plates incubated overnight at 37 °C were resuspended in sterile NaCl and adjusted to the 0.5 McFarland standard. The inoculum prepared above is diluted to 1×10^6 CFU/mL. Determination of minimum inhibitory concentrations (MIC) was performed following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines using the broth dilution method in a 96-well microtiter plate. 100 μL of the initial concentration extract was added to the first row of the microtiter plate. 50 μL of MHB was added to all wells in a row. After that, 50 μL of the extract was transferred from the first well to the second well and so on. In this way, the extract was diluted twice. 50 μL of bacterial suspension was added to all wells except the last (negative control). The next day, the activity of the extracts was read. The last well in which turbidity did not occur was read as the minimum inhibitory concentration of the extract.

Results and discussion

The content of total phenols, flavonoids and antioxidant activity of the leaf extracts are given in the Table 1. The content of extracted polyphenols and flavonoids as well as the antioxidant activity of leaf extracts depends on the applied solvent and method of extraction.

The content of total phenols in leaf of *Zizyphus jujuba* ranged from 22.71 to 28.69 mg GAE/g. The highest content of total phenols was in the extract obtained by maceration with 50% ethanol as a solvent (28.69 mg GAE/g) and infusion 100 °C (26.80 mg GAE/g). The total content of flavonoids ranged from 5.15 mg RE/g (ultrasonic extraction with 50% ethanol) to 8.25 mg RE/g (maceration with water). Phenolic compounds and flavonoids are well known for their free radical scavenging (antioxidant) activities (Đurović et al., 2023).

Table 1. Phytochemical content and antioxidant activity of leaf of *Zizyphus jujuba*

Leaf	Total phenols mg GAE/g	Total flavonoids mg RE/g	DPPH % inhibition	ABTS % inhibition
Infusion 80 °C	25.26±1.30	8.23±0.33	76.80±2.28	99.52
Infusion 100 °C	26.80±1.32	7.70 ±0.33	74.75±0.09	99.13
Maceration water	24.05±0.38	8.25±0.25	80.36±0.63	99.18
Maceration 50% etanol	28.69±0.36	7.70±0.20	86.34±0.15	99.98
Ultrasonic extraction - water	22.97±0.36	7.55±0.34	82.50±1.15	99.62
Ultrasonic extraction - 50% ethanol	22.71±0.19	5.15±0.21	87.48±0.46	99.66

All extracts showed a high level of inhibition and had strong free radical scavenging activities. The reason may be attributed to the differences of their phenolic contents and flavonoids contents. Various antioxidant activity assays such as the (DPPH) scavenging activity and ABTS assays were used to evaluate the effect of extraction method and solvents on the antioxidant activity in the leaf. These findings agree well with previous reports indicating that leaves of the *Ziziphus genus* showed a high antioxidant activity due to their high bioactive compounds content such as polyphenols including tannins and flavonoids (Esteki et al., 2012; Hossain et al., 2016).

Many studies have reported the impact of different solvents on the content of secondary metabolites and/or their antioxidant activity (Khaou et al., 2013; Ngo et al., 2017). In addition, the extraction techniques also have noticeable effect on the recovery of phytochemical content.

Antibacterial activity

Plants are a rich source of compounds that do not appear essential for primary metabolism, including thousands of secondary metabolites. In addition to their function in physiology or in structural maintenance, some of the secondary metabolites (e.g. flavonoids, anthocyanins, monoterpenes and tetraterpenes) serve for defense against microbes (Wink, 2008).

As shown in Table 2, the antibacterial activity of *Ziziphus jujuba* leaf extract was tested against three Gram-positive (*Staphylococcus aureus*, *Listeria monocytogenes* and *Listeria ivanovii*) and three Gram-negative (*Salmonella typhimurium*, *Salmonella enteritidis* and *Pseudomonas aerogenes*) bacteria.

The results of the antibacterial activity of 50% ethanol *Ziziphus jujuba* leaf extracts show that the tested extracts have an antibacterial effect. The leaf extract acted in concentrations of 25 to 50 mg/mL (*Salmonella typhimurium* and *Salmonella enteritidis* – 25 mg/mL) and on other tested bacteria (*Listeria ivanovii, Listeria monocytogenes, Staphylococcus aureus, Pseudomonas aerogenes*) in concentrations of 50 mg/mL.

Table 2. Minimum inhibitory concentrations (MIC) of ethanolic extract of *Ziziphus jujuba* leaf

Bacteral Spp.	MIC of leaf extract (mg/mL)
Listeria ivanovii	50
Listeria monocytogenes	50
Salmonella typhimurium	25
Staphylococcus aureus	50
Pseudomonas aerogenes	50
Salmonellla enteritidis	25

The results of this work were in agreement with Ghazghazi et al. (2014), who evaluated the antibacterial potential of jujube extract on several Gram-negative and Gram-positive bacteria (S. *typhi, E. coli, S. aureus*, and *B. cereus*). The antimicrobial effect is related to the ability of the compounds based on shape and size to penetrate into the outer membrane and reach their site of action (Kavak et al., 2010). The bactericidal effect depends on the concentration of active components in extracts and type of bacterial strains.

Conclusion

The results of the present investigation demonstrated the effects of extraction solvent and method on phenolic and flavonoid content and antioxidant activities of *Ziziphus jujuba* leaf. The results revealed that the extraction method greatly influences the phytochemical content and bioactivity and strongly recommends that any plant samples, intended to study, must undergo several extraction processes to reveal the actual phytochemical content. In this study, important phytochemical constituents were detected in both ethanol and aqueous leaf extracts of *Z. jujuba*. The phytochemicals detected in the plant extracts could be responsible for the observed antibacterial activities. The extracts exhibited antibacterial activities. Thus, further investigations should be carried out for individual phenolic compounds for potential medicinal and industrial uses. The toxicity profile of this plant should be carried out so as to establish its safety/therapeutic index.

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COMPARASION OF THE AROMA ACTIVE COMPOUNDS IN RAW SPIRITS OBTAINED FROM DIFFERENT APPLE VARIETIES

Milan P. Nikolić[1]

Abstract: Raw spirits were produced through distillation of apple wines obtained by fermentation of selected varieties of apples. Two types of apple wine were obtained using different apple varieties. In the first case, the wine was obtained from two types of apples: Red Elstar and Vilmuta. In the second case, the wine was obtained from mixture composed of four types of apples: Idared, Golden Delicious, Jonagold and Melrose. The aromatic profiles of obtained raw spirits were strongly influenced of desirable aromas of ethyl esters which contribute to the flavour of the destillates with a pleasant fruity and flowery smell, indicative of the quality of the spirit. A headspace solid phase microextraction (HS-SPME) as the extraction technique and gas chromatography coupled with mass spectrometry was utilized for the determination of volatile compounds. It was shown that raw spirits obtained from mixed combination of Red Elstar and Vilmuta have more intense aroma than obtained from second mixture (Idared, Golden Delicious, Jonagold and Melrose). Ethyl nonanoate, ethyl 9-hexadecanoate, ethyl palmitate and 9,12-octadecanoic acid ethyl ester were detected only in raw spirits obtained from Red Elstar and Vilmuta. The aroma profile of the fractions obtained during the distillation was also investigated. The most intensive aroma was detected in the first fractions (heads). On the other side, the content of ethyl esters in middle fractions was lower than that in the first fractions.

Keywords: raw spirits, apple varieties, ethyl esters, headspace solid phase microextraction

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Introduction

The presence, content and composition of volatile substances in agricultural distillates (raw spirits) have a substantial influence on their quality. The aroma of the raw spirit depends on the type and quality of the raw materials as well as the hygienic and fermentative conditions during processing. The volatile aroma was also influenced by the distilling stage (head, heart and tail) (Zheng at al., 2014). The chemical composition of volatile byproducts such as carbonyl compounds, alcohols, esters, acids, and acetals are often diverse and contribute to the peculiar flavor of the spirit. Esters are well-known aroma compounds occurring at relatively high content and exhibiting a low odor threshold and therefore are quite relevant for the beverage sensory properties (Nascimento et al., 2008; Plutowska et al., 2008).

Apple brandy is a spirit obtained by the distillation of cider or apple wine. The apples can be classified as table, commercial, or cider apples, but all are suitable for alcohol production (Kosseva, 2017). The volatile components of freshly distilled Calvados and Cognac have been extensively investigated, and 331 compounds, of which 162 can be considered as trace compounds, were characterized. The aroma quality of apple brandy is influenced by the cider maturation (Rodríguez-Madrera et al., 2010). It was found that the most mature cider gave a distillate of superior aroma (with more sweet and spicy character), with higher levels of ethyl acetate, ethyl lactate, and ethyl succinate, and volatiles derived from bacterial metabolism (which is more prevalent in extensively matured cider), such as 2-butanol, 4-ethylguaiacol, eugenol, and 2-propen-1-ol.

The aim of this study was to examine the effects of different types of apple varieties on aroma profiles of obtained raw spirits. A procedure for qualitative determination of fatty acid ethyl esters, in raw spirits of different quality or produced from various raw materials, by means of headspace solid phase microextraction (HS-SPME) as the extraction technique and gas chromatography with mass spectrometry (GC-MS) as the determination technique. These esters are known to be the most abundant esters in spirits, and their presence is commonly related to the pleasant fruity bouquet of alcoholic beverages.

Materials and methods

Raw spirits were obtained by one step distillation of apple wines obtained by fermentation of apple juice obtained from mixed varieties of apples: i) two types of apple varieties (Red Elstar and Vilmuta) were used at a weight ratio of 1:1; ii) four types of apple varieties (Golden Delicious, Idared, Melrose and Jonagold) at a weigt ratio of 1:0.9:0.6:0.5, respectively (Table 1). The apples were washed and then ground in fruit mill. Obtained fruit pulp was put on hydraulic press to separate fresh juice from solid part.

Apple juice was fed into 100 L stainless steel fermenter (Fig. 1) with self-contained cooling. The fermentation was performed at 17 °C.

Figure 1. Fermenter used for fermentation of apple juice (location: Laboratory for Unit Operation and Biochemical Engineering; Faculty of Agronomy, Čačak)

The aroma profile was collected using headspace solid-phase micro-extraction followed by gas chromatography (Agilent Technologies 7890 B GC System, AIM, Littleton, CO, USA) coupled with mass spectrometry (Agilent Technologies 5977A MSD, AIM, Littleton, CO, USA). Briefly, the 0.2 mL of the sample (having 20 vol% of ethanol) was placed in a headspace vial. Each vial was sealed using a cap with PTFE/silicone septa and incubated at 27°C. Octanoic acid-methyl ester was used as internal standard at a concentration of 500 µg/L. The solid phase microextraction fiber (Polydimethylsiloxane (PDMS) 100 µm, Agilent Technologies, AIM, Littleton, CO, USA) was inserted into the head space of the vial containing the sample solution. The extraction was carried out for 70 min of fiber-exposed time. After sampling, the SPME fiber was withdrawn into the needle, removed from the tube, and inserted into the hot injector port (270 °C) of the GC system where the extracted analyte was desorbed and transferred to the analytical column (HP-5, Agilent Technologies, AIM, Littleton, CO, USA). A relatively long desorption time in the injector (10 min) was selected to avoid carryover between runs to ensure full desorption of analyte from the fiber. Ultra-high purity 5.0 grade helium (Messer Tehnogas AD, Belgrade, Serbia) was used as a carrier gas at a flow rate at 1.2 mL/min along with the spitless injection. The oven temperature was programmed for an initial 30 ℃ for 5 min and was then increased in four steps: 30–40 °C at a rate of 3 °C/min and held for 2 min at this temperature; 40–100 °C at a rate of 5 °C/min, 100–180 °C and held for 2 min at this temperature; 180-280 °C at a rate of 25 °C/min, and held 1 min.

Volatile compounds were identified by comparison with the National Institute of Standards and Technology (NIST) database. The VOCs that showed mass spectra with match factor ≥80% were considered as identified substances.

Results and discussion

Fig. 1 shows GC profile of volatile organic compounds (VOCs) in raw spirits (heart) obtained from Red Elstar and Vilmuta (a) and Golden Delicious, Idared, Melrose and Jonagold (b). On the other side, the list of detected volatile organic compunds of chromatogram 2-a are shown in table 1.

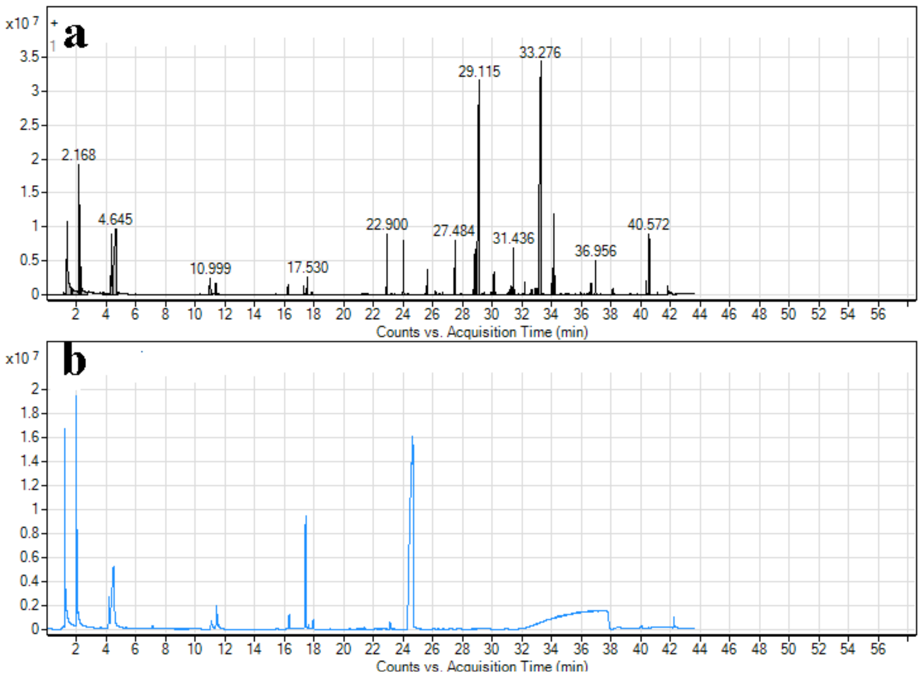

Figure 2. GC profile of volatile organic compounds (VOCs) in raw spirits (heart): a) the VOCs profile in distillate obtained from Red Elstar and Vilmuta; b) the VOCs profile in distillate obtained from Golden Delicious, Idared, Melrose and Jonagold.

Table 1. List of detected volatile organic compunds in raw spirit obtained from Red Elstar and Melrose

No	compounds	Retention time (min)	No	compounds	Retention time (min)
1.	ethanol	1.36	16	Isoamyl caprylate	30.1
2.	ethyl acetate	2.16	17	Octanoic acid, 2-methylbutyl ester	30.18
3	Isobutanol	2.3	18	Propyl decanoate	31
4	Acetal	4.33	19	α-Farnesene	31.4
5	Isopentyl alcohol	4.58	20	n-Capric acid isobutyl ester	32.16
6	Butyraldehyde, diethyl acetal	10.3	21	Octanoic acid, hexyl ester	32.9
7	1-Hexanol	10.98	22	Ethyl laurate	33.2
8	Isopentyl alcohol, acetate	11.38	23	Iso-Amyl n-decanoate	34.13
9	Ethyl caproate	17.3	24	Propyl dodecanoate	34.95
10	Hexyl acetate	17.85	25	Isobutyl laurate	35.95
11	Ethyl caprylate	24	26	Ethyl myristate	36.95
12	Acetic acid, phenethyl ester	25.6	27	Isoamyl laurate	38.1
13	Ethyl nonanoate	26.64	28	Ethyl 9-hexadecanoate	40.3
14	Ethyl trans-4-decanoate	28.82	29	Ethyl palmitate	40.5
15	Ethyl caprate	29	30	9,12-Octadecadienoic acid, ethyl ester	41.8

As it was shown in Fig 2. and table 1, raw spirits obtained from Red Elstar and Vilmuta have more intense aromas than that obtained from mixed varieties of Golden Delicious, Idared, Melrose and Jonagold. Chromatogram 2a shows the presence of some ethyl-esters (ethyl nonanoate, ethyl 9-hexadecanoate, ethyl-palmitate, 9,12-octadecanoic acid ethyl ester) which were not detected in the sample obtained from mixed varieties of Golden Delicious, Idared, Melrose and Jonagold.

Fig. 3 shows aroma profiles of the fractions obtained during the distillation of apple wine obtained from mixed varieties of Golden Delicious, Idared, Melrose and Jonagold. Internal standar (octanoic acid-methyl ester, at concentration of 500 µg/L) was detected at retention time of 22 minute. The most intensive aroma was detected in the first fractions (heads), however, there were undesirable components such as hydrocyanic acid. This indicates that alcohol-ester azeotrope and an ester-water azeotrope may be formed during distillation which allows esters evaporation at lower distillation temperatures. On the other side, the content of ethyl esters in middle fractions was lower than that in the first fractions. The lowest aroma profile was detected in at the end of distillation run (tails).

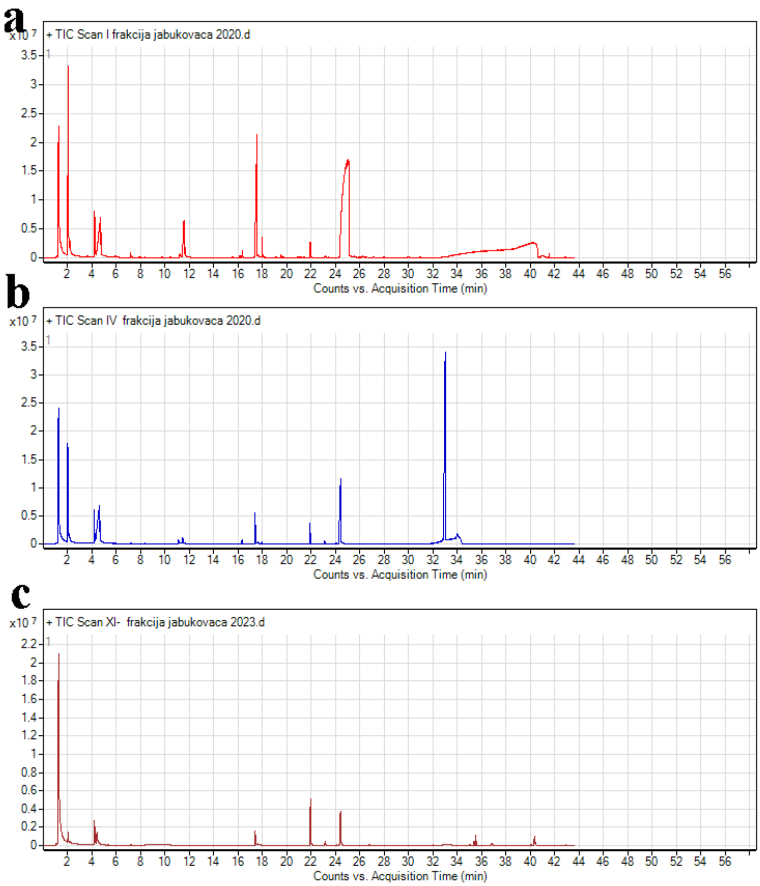

Figure 3. The volatile aroma profile at different the distilling stage: a) head, b) heart, c) tail.

Conclusion

Raw spirits were produced through distillation of apple wines obtained by fermentation of selected varieties of apples. The aromatic profiles of obtained raw spirits were strongly influenced of desirable aromas of ethyl esters which contribute to the flavour of the destillates with a pleasant fruity and flowery smell, indicative of the quality of the spirit. Aroma profile of raw spirit obtained from Red Elstar and Vilmuta have more intensive aroma profile than that obtained from Golden Delicious, Idared, Melrose and Jonagold. The presence of some esters including ethyl nonanoate, ethyl 9-hexadecanoate, ethyl-palmitate, 9,12-octadecanoic acid ethyl ester were detected in raw spirit obtained from Red Elstar and Vilmuta. The most intensive aroma profile was detected in the first fractions (heads) probably due to formation alcohol-ester azeotrope and an ester-water azeotrope. It was shown that solid phase microextraction coupled with gass chromatography/mass spectrometry allows qualitative analysis of aromas in raw spirits of different organoleptic quality. High precision and simple sample preparation enable the use of this method for routine investigations in both industrial and research laboratories.

Acknowledgement

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AUTOMATIC CONTROL AND ARTIFICIAL INTELLIGENCE: DEVICE FOR CONTINUOUS CHLORINE RESIDUAL MEASUREMENT

Tomislav Trišović[1], Ana Trišović[2], Lidija Rafailović[3], Branimir Grgur[4], Zaga Trišović[5]

Abstract: Bacteriologically safe water is crucial for potable and food production, which is obtained using disinfection. Disinfectants must be broad-spectrum, act swiftly, withstand organic matter, maintain stability across pH levels, be non-toxic, minimally corrosive, and pleasant-smelling. Precise dosing is vital, typically achieved via automated membrane dosing pumps, and is ensured by measuring residual chlorine post-disinfection. This paper introduces devices utilising amperometric electrodes to maintain active chlorine levels of 0.2 to 0.5 mg/l. Industrial computer and touch panel aid data storage and visualisation, enabling performance prediction via machine learning. Deployed at the dairy company Kozanostra in Osečina, it's a major water safety advancement.

Keywords: water quality, disinfection, automation, machine learning, artificial intelligence

Introduction

Drinking water must be free from pathogenic microorganisms in order for it to be safe for consumption. As most water sources fall short, it's necessary to implement appropriate measures to ensure water becomes bacteriologically

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safe. Continuous disinfection is crucial, preventing waterborne diseases like typhoid, paratyphoid, dysentery, etc. Continuous disinfection is essential for well water, springs, reservoirs, swimming pools, septic tanks, industrial wastewater outflows, and sewage. Different water supply systems require specific disinfection devices. Chemical disinfectant addition generally involves gravitational drainage or pressurised methods. Amperometry, an electrochemical technique, measures hypochlorite ion reduction to regulate disinfectant dosage for maintaining optimal levels. This method directly measures free chlorine concentration and incorporates a continuous hydraulic cleaning system to minimise calibration needs, ensuring efficient operation. The limiting current of reduction on the rotating disk electrode can be calculated from the following equation:

$$j_g = 0.620 z F C_O D^{2/3} \nu^{-1/6} \varpi^{1/2}$$

where ϖ is angular velocity, ν is kinematic viscosity, D is the diffusion coefficient, C_O is concentration of hypochlorite at the depth of the solution, z is number of exchanged electrons in the reaction, and F is Faraday's constant, which is $9{,}65 \cdot 10^4$ C/mol.

Also, an essential component is a measuring probe, which is a cell responsible for assessing residual chlorine levels post-dosing and then regulating dosing intensity after. The addition of disinfectants, mostly chlorine, into the pipeline is carried out by dosing pumps or injectors. The residual chlorine values are maintained at from 0.2 to 0.5 mg/l.

In the case of the presence of nitrogen compounds such as ammonia in the water, free chlorine reacts with them to form chloramines (monochloramine NH_2Cl, dichloramine $NHCl_2$, trichloramine NCl_3), which are called combined chlorine (bound chlorine). The sum of free and combined chlorine constitutes total chlorine.

Equipment for automatic control of the water disinfection process consists of the following elements: inlet pipeline, equalisation tank (hydropneumatic tank), outlet pipeline, measuring element - sensor on the outlet pipeline, controller that converts the measured value from the sensor into an electrical signal and activates and deactivates the actuator (dosing pump) that introduces hypochlorite into the inlet pipeline. The block diagram of such a device with negative feedback where the system output follows the specified input parameters is shown in Figures 1.

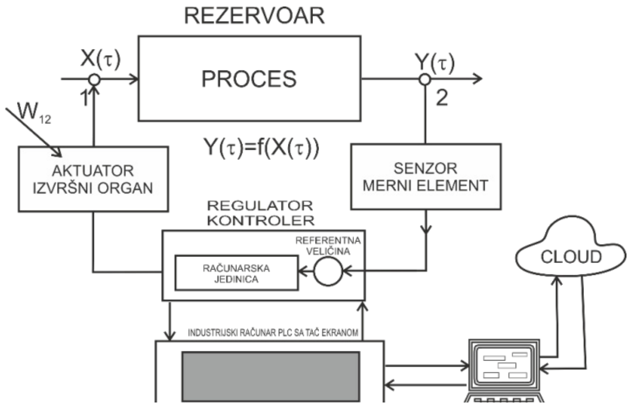

Figure 1. Block diagram of a negative feedback control system

The device for measuring chlorine content in water is used to measure and store the data in a database. Based on the operational history of measured parameters over an extended period of several months, it is possible, with the help of machine learning and neural networks, to forecast the operation of such systems in the future. The block diagram of such a system is given in Figure 2.

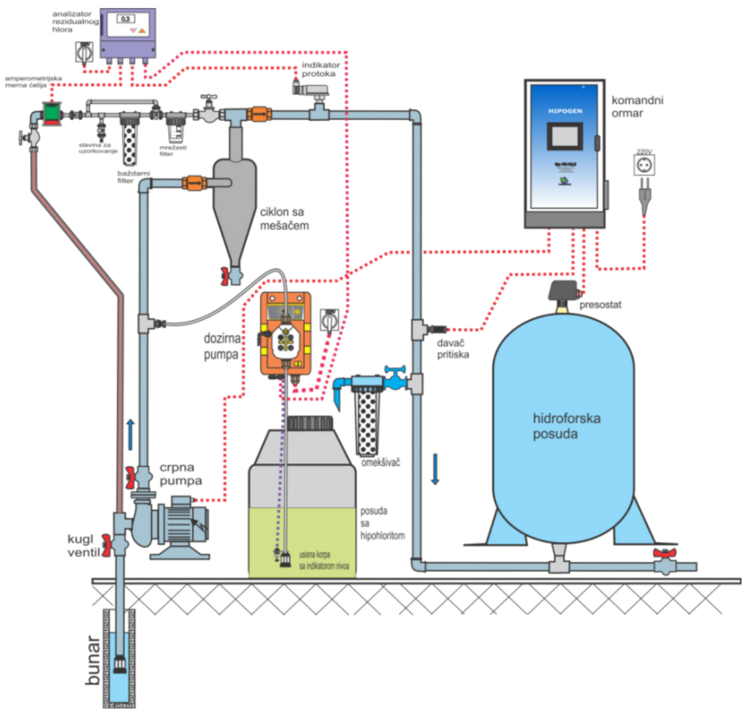

Figure 2. Water disinfection system with operational history and AI

Materials and methods

The device for measuring chlorine content in water consists of: Controller LDSCL (ERMES COMMUNICATION), LCD monitor WEINTEK cMT2078X, LC controller DELTA DVP-14SE. The LDSCL controller measures chlorine levels in water from 0 to 10 mg/l, displayed on an LCD screen. Adjustments are made via a multifunctional button. Housed in a plastic IP65-rated enclosure, it transmits data to an LCD HMI monitor via RS485, ModBUS RTU. Values are stored in the monitor's memory and can be viewed locally or on a PC via ETHERNET. Communication with the PLC controller activates the electromagnetic valve for water flow through the sensor.

The screen displays chlorine content and valve status (green "light" VALVE). Data can be viewed in tabular or graphical form, accessed via "GRAPHICAL VIEW" or "TABLE VIEW" buttons, as shown in Figure 3. The valve opens hourly from 5:00 AM for 10 minutes. Measurements are recorded every minute for 5 minutes after the flow begins. Data in the table includes measurement number, time, date, and chlorine content.

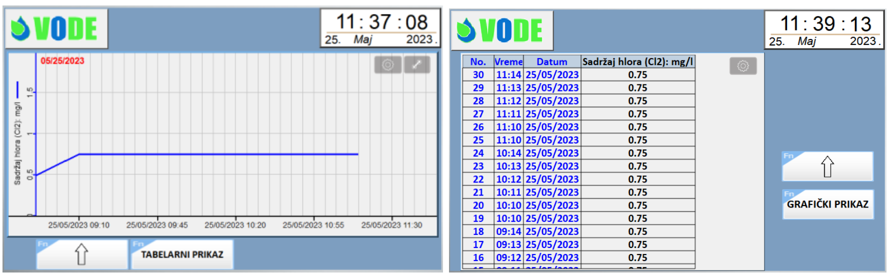

Figure 3. Graphical (left) and tabular (right) representations of obtained results

The LCD monitor can be connected to a PC in a local network. To establish communication between the computer and the LCD HMI monitor, the devices must be connected with a network cable with RJ45 connectors at both ends, and the IP addresses of both devices must be set in the same address range. The cMTViewer program needs to be installed on the PC. By running the program and configuring the IP address on the monitor, the PC will display the figure from the LCD HMI monitor.

Once communication between the LCD HMI monitor and the PC is established, all screens can be displayed on the PC just like on the LCD HMI monitor. Files with the archive of measured values can be copied from the PC to the remote computer.

Figure 4. Device for measuring residual chlorine with automatic control and AI

Results and discussion

The study analyses chlorophyll concentrations empirically using a dataset of 21,271 observations spanning from June 2023 to February 2024. The data undergoes preprocessing to normalise values and refine temporal granularity to 12-hourly averages.

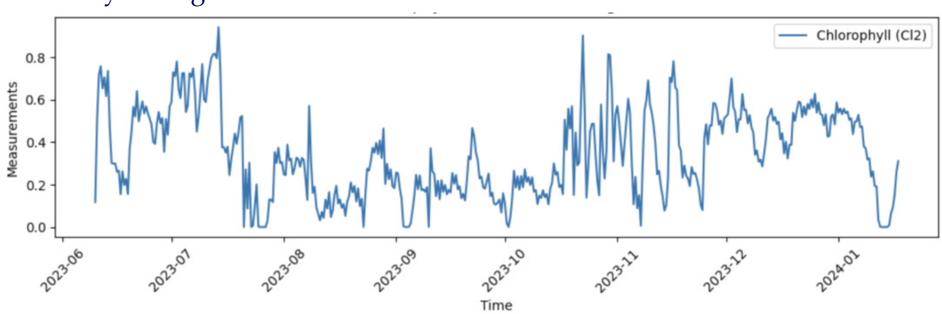

Figure 5. Chlorophyll Cl_2 (mg/l) over time

Three models are applied: Long Short-Term Memory (LSTM), Gradient Boosting Regressor, and Autoregressive Integrated Moving Average (ARIMA). They are evaluated based on predictive accuracy and ability to capture temporal patterns.

Table 1. Model Performance Comparison

Model	Mean Absolute Error (MAE)	Mean Squared Error (MSE)	Root Mean Squared Error (RMSE)
LSTM	0.1725	0.0377	0.1943
Gradient Boosting	0.0913	0.0197	0.1403
ARIMA	0.0747	0.0125	0.1118

In chlorophyll concentration forecasting using time series data, ARIMA model demonstrates superior performance, with lowest MAE, MSE, and RMSE values, indicating effective capture of temporal patterns. Gradient Boosting also performs well in handling non-linear patterns, surpassing LSTM. The latter's lower performance suggests complexity and potential overfitting issues. These findings stress the importance of model selection tailored to dataset characteristics. For this task, ARIMA's focus on temporal patterns yields the most reliable predictions.

Conclusion

Based on the presented results, it can be concluded that:

1. The device for continuous measurement of residual chlorine with automatic control and artificial intelligence is a functional solution and has been operating in exploitation for several months.
2. Chemical analysis of the outlet water shows that the quality of the obtained water meets the Regulations on the Chemical and Bacteriological Potability of Drinking Water and that the water can be used in households.
3. Thanks to machine learning and neural networks, the device predicts the future operations of the system and informs the user about future material and energy requirements necessary for the device operation.
4. With the bacteriologically and chemically safe water, and continual monitoring system of the drinking water quality, the dairy company

Kozanostra in Osečina has acquired the necessary conditions for exporting its products like cheese, yogurt, and powdered whey.

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QUALITY PARAMETERS OF SILAGE MAIZE HYBRIDS FOR RUMINANT FEED

Danka Milovanović[1], Beka Sarić[1], Valentina Nikolić[1], Marijana Simić[1], Slađana Žilić[1]

Abstract: Silage is a byproduct of carefully regulated fermentation that takes place in anaerobic environments where oxygen is absent and is fed to farm animals. When it comes to biomass quality, yield, ensiling suitability, and variety of uses in domestic animal diets, maize is the most significant fodder plant. Four commercial hybrids from the Maize Research Institute Zemun Polje were investigated in this study at four separate locations in Serbia. The amount of lignocellulosic fibers, dry matter content, and digestibility of the whole hybrid maize plant were evaluated. Based on the obtained results of ZP, corn hybrids are characterized by high digestibility of dry matter of the whole corn plant.

Keywords: *in vitro* digestibility, maize, ruminant feed, silage quality

Introduction

One of the main sources of forage and energy for livestock production is whole plant maize silage. In the Republic of Serbia, maize (*Zea Mays* L.) accounts for 53% of the harvested area under cereals, making it the most common cereal crop (Grčak et al., 2020). The fact that practically all of the plant's above-ground biomass can be utilized is the indication of the unique economic significance of maize. The whole maize plant is used to produce over 1,600 different industrial products. In livestock production, above-ground biomass, either fresh or used to make silage, comes next. The whole grain of maize is used as concentrated fodder for feeding farm animals (Popović, 2010). Based on statistical data, 2,711 hectares of silage maize were planted in Serbia in 2021 (Statistical Yearbook of the Republic of Serbia, 2022). Hybrids of different subspecies of maize are also intriguing to small farmers because their grain is becoming a more significant part of peoples' diets. Silage preparation is a traditional farming method that dates back more than 3,000 years. Silage was first used to preserve animal feed. The development of machinery for forage collection led to a wider application of the new forage preservation technology

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after the turn of the 19th century. Today, ensilaging is a commonly used method of animal feed preservation worldwide. The benefit of using silage plant mass is that forage plants can be harvested at the ideal time of development and keep them segregated until they are needed. Silage is made by fermenting soluble carbohydrates, which results in the production of acids that protect the fodder. Silage offers several benefits over animal fodder, including superior quality, high digestibility, and good palatability (Coblentz and Akins, 2018). These days it is difficult to imagine intensive livestock production without the use of silage storage. Silage of green biomass from fodder plants allows for more intensive land use and their early removal from production areas (Rakašćan et al., 2019). The fact that the initial silage material remains mostly unchanged following the acidification of the green mass via natural fermentation processes or the addition of specific additives is indicative of the significance of silage. When it comes to livestock nutrition, silage can completely replace fodder. As the most direct alternative to fodder, silage feeding is considered the cornerstone of contemporary, economically sound animal husbandry (Đorđević et all., 2011). The aim of this work is to determine the quality parameters of silage corn hybrids.

Materials and methods

Five ZP maize hybrids of the FAO 700 maturity group created at the Maize Research Institute Zemun Polje were tested for quality parameters in the Maize Research Institute's Group for Food Technology and Biochemistry laboratory in order to assess the hybrids' potential for silage preparation. Four distinct locations in Serbia were selected for silage maize hybrid field experiments, namely: Žarkovac in Srem, and three locations in Central Serbia - Murgaš, Valjevska Loznica and Donja Trepča. Hybrid 7001 was utilized as a standard in addition to the four tested hybrids. Along with determining the total dry matter content and *in vitro* digestibility of the maize plant, also measured the amount of lignocellulosic fibers present. According to the detergent method of Van Soest (1980), with minor modifications, the fiber analysis comprised identification of lignocellulosic constituents: neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL), hemicellulose, and cellulose (Mertens, 1992). The studied maize hybrid whole plant samples' *in vitro* dry matter digestibility was evaluated using Aufrere's (2007) enzymatic pepsin-cellulase technique. The results are shown as percentages of dry matter (d.m.).

Results and discussion

The main nutritional benefit of maize is its high carbohydrate content given that it has low protein and mineral content. Data on the *in vitro* digestibility of dry matter and the fractions of lignocellulosic fibers (NDF, ADF, ADL, hemicellulose, and cellulose) are required for a more accurate definition of the nutritional value of coarse nutrients. When selecting individual lines to create hybrids for silage that will eventually find a home on the domestic and international markets, breeders should find assistance from these quality parameters. Table 1. shows the amount of lignocellulosic fibers in each location throughout the tested maize hybrid plants.

Table 1. The content of lignocellulosic fibers in the whole plant of the tested maize hybrids, by location

Location	Hybrid	The content of lignocellulosic fibers				
		NDF	ADF	ADL	Hemicellulose	Cellulose
Žarkovac (Srem)	7001std.	54.11	26.51	2.80	27.61	23.71
	ZP 707	51.62	23.33	2.48	28.28	20.86
	ZP 7357	51.33	26.52	3.36	24.81	23.16
	ZP 7777	53.40	25.41	2.94	27.98	22.47
	ZP 7900	49.83	24.46	2.84	25.37	21.62
Murgaš (Central Serbia)	7001	58.42	24.13	2.33	34.29	21.81
	ZP 707	53.34	21.31	2.04	32.04	19.26
	ZP 7357	48.31	24.69	3.37	23.91	21.32
	ZP 7777	54.53	26.68	3.27	27.78	23.48
	ZP 7900	54.08	25.95	3.37	28.13	22.58
Valjevska Loznica (Central Serbia)	7001	45.37	19.82	2.31	25.55	17.51
	ZP 707	51.12	24.43	3.06	26.68	21.38
	ZP 7357	42.46	18.69	2.28	23.77	16.41
	ZP 7777	49.00	23.74	3.15	25.76	20.59
	ZP 7900	39.69	18.76	2.38	20.93	16.37
Donja Trepča(Central Serbia)	7001	54.92	24.61	1.99	30.32	22.61
	ZP 707	50.76	23.37	1.81	27.39	21.56
	ZP 7357	52.99	26.21	2.28	26.78	23.93
	ZP 7777	50.09	24.96	2.65	25.13	22.32
	ZP 7900	59.89	22.67	4.13	26.50	29.45

Taking into account the outcome of domestic animals' nutrient utilization is vital to evaluate the nutritional value of maize plants as feed. Nutrient digestibility tests are performed for this reason, and the results show how well the nutrients are being utilized. Digestion-based hybrid evaluation is a more unbiased and trustworthy method of comparison. Given that the digestibility of cellulose-containing plant parts is genetically based, analyses of the in vitro digestibility of the whole and morphological fractions of the maize hybrid plant are especially important because there are notable variations in digestibility (Demirel et al., 2011). Given that digestibility is unaffected by energy density, it is becoming more and more crucial to examine it when it comes to domestic animal nutrition. Table 2. shows the content of dry matter as well as the digestibility of dry matter of the whole plant.

Table 2. Dry matter content and digestibility of the whole maize hybrid plant, by location

Location	Hybrid	Dry matter content (%)	*In vitro* dry matter digestibility (%)
Žarkovac (Srem)	7001 std.	44.16	50.33
	ZP 707	51.30	56.64
	ZP 7357	59.02	53.05
	ZP 7777	54.49	56.51
	ZP 7900	64.83	59.82
Murgaš (Central Serbia)	7001 std.	45.52	57.64
	ZP 707	47.38	62.95
	ZP 7357	63.09	61.13
	ZP 7777	52.38	57.62
	ZP 7900	69.09	59.26
Valjevska Loznica (Central Serbia)	7001 std.	50.47	64.17
	ZP 707	47.83	59.64
	ZP 7357	48.84	68.41
	ZP 7777	46.21	60.71
	ZP 7900	49.19	67.71
Donja Trepča (Central Serbia)	7001 std.	40.73	58.96
	ZP 707	37.40	64.89
	ZP 7357	34.57	61.01
	ZP 7777	34.72	64.27
	ZP 7900	34.93	53.75

Agrometeorological conditions, hybrid selection, sowing density, harvest time, and swath height all affect the quantity and quality of maize biomass produced (Ayub et al., 2011). The selection of hybrids has a significant impact on the quality and yield of maize biomass. Whether the hybrid will be grown as a main crop or a secondary crop is taken into consideration when choosing the hybrid, as well as the altitude at which it will be grown. Studies have indicated a discernible pattern in the rise in the dry matter yield when the FAO group of maize hybrids ripens. For instance, FAO group 700 exhibited a yield that was ten tons per hectare greater than FAO group 200. No compensatory differences in yield between hybrids of early and late FAO ripening groups were observed with higher sowing densities of earlier ripening group hybrids. For silage production, maize hybrids should be sown at an ideal density that is 5–10% higher than that of grains. Silage maize should be harvested at the point of physiological maturity, which occurs when the plant's dry matter content ranges from 35 to 42% (full waxy maize maturity). For silage maize, a swath height of 20 cm is ideal. Tables 3 and 4 show the average values of in vitro digestibility and dry matter content of the whole plant, both by hybrids and by location, as well as the coefficient of variation.

Table 3. Average values of *in vitro* digestibility and dry matter content of the whole plant by hybrids for all locations and coefficient of variation

Hybrid	Dry matter content (%)	CV (%)	*In vitro* dry matter digestibility (%)	CV (%)
7001 standard	45.22±4.03	8.93	57.78±5.71	9.88
ZP 707	45.98±5.98	13.01	61.03±3.64	5.97
ZP 7357	51.38±12.71	24.73	60.90±6.27	10.30
ZP 7777	46.95±8.88	18.91	59.78±3.48	5.82
ZP 7900	53.01±13.94	26.39	60.13±5.74	9.55

Table 4. Average values of *in vitro* digestibility and dry matter content of the whole plant for each location for all hybrids and coefficient of variation

Location	Dry matter content (%)	CV (%)	*In vitro* dry matter digestibility (%)	CV (%)
Žarkovac	54.76±7.81	14.26	55.27±3.65	6.61
Murgaš	54.24±8.41	15.49	59.71±2.31	3.87
Valjevska Loznica	48.51±1.59	3.28	64.13±3.97	6.18
Donja Trepča	36.30±2.79	7.69	60.57±4.51	7.46

In terms of quality parameters for maize silage production, the best hybrids were ZP 7357, ZP 7900, ZP 7777, and ZP 707. In most cases, all investigated hybrids performed better than the standard. Regarding digestibility, the relative stability by location was a characteristic shared by all tested hybrids. Whereas ZP 7357 varied the most in terms of digestibility and dry matter content, ZP 7001 was found to be the weakest hybrid overall. Higher amounts of lignin (ADL), ADF, and cellulose had a detrimental effect on the plant's overall digestibility. Despite having the highest dry matter content overall, the hybrids at the Žarkovac ZP location had the lowest digestibility. The lowest dry matter content was achieved by all tested hybrids at the Donja Trepča location.

Conclusion

Based on the obtained results, the Maize Research Institute, Zemun Polje silage ZP maize hybrids are distinguished by a high yield of digestible dry matter and total dry matter per hectare, a high proportion of cob dry matter in the total dry matter yield, and a high digestibility of the dry matter of the whole maize plant. When compared to hybrids chosen by both domestic and international selection firms, their quality is competitive.

Acknowledgement

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OPTIMIZATION OF ULTRASONIC-ASSISTED EXTRACTION OF PHENOLIC COMPOUNDS FROM *SALVIA VERTICILLATA* L. ROOT USING RESPONSE SURFACE METHODOLOGY

Nevena Mihailović[1], Andrija Ćirić[1], Nikola Srećković[1], Vladimir Mihailović[1], Ljubinka Joksović[1], Samo Kreft[2]

Abstract: This study aimed to optimize the conditions of ultrasonic-assisted extraction to obtain the highest content of phenolic compounds from *Salvia verticillata* L. root using response surface methodology. RSM was applied to maximize total phenolic content from *Salvia verticillata* L. root while minimizing operative temperature, ethanol composition, the solvent-to-solid ratio, and time. The obtained optimal conditions were ethanol percentage of 20 %, solvent-to-solid ratio of 40 mL g^{-1}, a temperature of 33.68 °C, and a time of extraction of 10 minutes. Obtained optimal conditions could be used for further investigation of the biological activities of *Salvia verticillata* L. and related plant species.

Keywords: *Salvia verticillata* L., ultrasonic extraction, computer optimization, total phenolic content, response surface methodology

Introduction

The extraction procedure is one of the most essential steps in the determination, separation, and identification of compounds in analyzed plant materials. The use of ultrasound for phenolic compound extraction has been reported as a faster, solvent-saving, and more efficient technique than traditional extraction procedures (Tao et al., 2014). A cavitation effect generated by ultrasonic waves may accelerate the release of target compounds and speed up the extraction process. Several factors, including temperature of extraction, used solvent composition, extraction time, and solvent-to-solid ratio may impact the ultrasonic-assisted extraction (UAE) efficiency (Zhou et al., 2017).

Salvia verticillata L. is (lilac sage) belongs to the genus *Salvia* (Lamiaceae). Salvia plants have applications in the food, aromatic, and pharmaceutical

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industries. *Salvia verticillata* L. is traditionally used as an expectorant, for disinfection of the oral cavity, and wound healing (Jarić et al., 2015). *S. verticillata* contains polyphenols and diterpenoids and is recognized for its antioxidant properties. According to certain studies, *S. verticillata* is a significant source of rosmarinic and salvianolic acid and its derivatives (Šulniūtė et al., 2017). Several papers have mentioned the plant's biological activity, including its antibacterial and anti-diabetic properties, but also the potential of being used in lost or declining cognitive functions (Naderi et al., 2011).

Considering the potential of this plant and bearing in mind that the *S. verticillata* root research is negligible compared to the aerial part, the idea of this study was to find the optimal conditions for ultrasonic-assisted extraction of *S. verticillata* root, in order to maximaze the content of total phenolic compounds, while utilizing a minimal amount of solvent and short extraction time, all while maintaining the lowest possible extraction temperature. The obtained results can be applied to additional evaluations of antimicrobial activity, antioxidant activity, phenolic composition, etc.

Materials and methods

Chemicals and plant material. Folin-Ciocalteu reagent used for the determination of total phenolic content was purchased from Sigma Aldrich (Deisenhofen, Germany). Root parts of *S. verticillata* were collected in October 2017, at the locality near Kragujevac, Central Serbia. The identification of species was carried out at the Herbarium of the Department of Biology and Ecology, Faculty of Science, University of Kragujevac (Kragujevac, Serbia). After being cleaned of impurities, plant portions were placed in a dark, well-ventilated room to dry. The dry root parts were finely powdered and 1 g of each was measured.

Software. The experimental design, data analysis, and desirability function calculations were performed using Design Expert trial version 13.0 (Stat-Ease Inc., Minneapolis, MN, US).

Preparation of the *S. verticillata* extracts and experimental design for extraction optimization. Samples (1 g) were extracted using ethanol : water solvent with varying ethanol composition (20, 60, 100, v/v%) at different temperatures (25, 50, 75 °C) and for different extraction times (10, 20, 30 min) in an ultrasonic bath (Bandelin Sonorex RK 52 H, Bandelin electronic GmbH & Co. KG, Berlin, Germany) which was coupled with circular thermostat (Lauda Alpha A6, LAUDA-Brinkmann, Delran, NJ, USA) to keep the temperature constant and prevent overheating.

Spectrophotometric determination of phenolic compounds. The Folin-Ciocalteu procedure was applied to determine the total phenolic content in *S. verticillata* extracts (Singleton et al., 1999). Plant extracts were diluted with methanol to a concentration of 0.4 mg mL^{-1}, and 1.25 mL of Folin-Ciocalteu reagent (previously diluted ten-fold in water) was mixed with 1 mL of 7.5% aqueous NaHCO3 solution and added in 0.25 mL of extracts. This reaction mixture was incubated at 45°C for 15 min and the absorbance was measured at 765 nm. Known concentrations of gallic acid were used to obtain a standard curve, and the results were expressed in milligrams of gallic acid equivalents (mg GAE g^{-1} dry weight of extracts).

Results and discussion

Four extraction variables (solvent volume, ethanol composition, sonication time, and extraction temperature) were optimized by statistical experimental design to produce the best yields of total phenolics. The recovery of phenolics is influenced by the temperature, duration, and solvent-to-solid ratio during extraction, resulting in inconsistent outcomes regarding the solubilization and breakdown of phytochemicals (Robards, 2003).

Optimization of the extraction procedure was performed by response surface methodology (RSM) with central composite design (CCD). The CCD experimental design is given in Table 1. Solvent-to-solid (range 10-40 mL g^{-1}), ethanol concentration (20-100%), time (10-30 min), and temperature (25-75 °C), and central points were selected based on the initial experimental results and literature data. The independent variables are coded at three levels (–1, 0, and +1) and the experimental design consisted of 30 experimental points.

The selection of the best conditions for the extract preparation was carried out using multi-response analysis following the methodology presented by Derringer & Suich (Derringer and Suich, 1980).

The response in the experimental design was calculated as the total amount of phenolics, and its range was 5.41 – 58.2 mg GAE g^{-1} dry weight of *S. verticillata* root. Taking into consideration the range of input variables, and in order to obtain the maximum quantity of phenolic compounds from the extract, the optimal conditions were determined - ethanol percentage of 20 %, solvent-to-solid ratio 40 mL g^{-1}, temperature of 33.68 °C and extraction time of 10 minutes, with desirability of 0.862. Total phenolic content obtained under optimal conditions was 40.79 mg GAE g^{-1} DW of *S. verticillata* root.

Table 1. Central composite design (CCD) with the responses of the dependent variables

Run	EtOH (%)	Solvent-to-solid (mL g^{-1})	Time (min)	Temperature (°C)	Total phenolics (mg GAE g^{-1} DW)
7	20	10	10	25	5.91951
24	100	10	10	25	12.1549
23	20	40	10	25	34.2679
4	100	40	10	25	21.3958
16	20	10	30	25	5.41009
14	100	10	30	25	14.4381
18	20	40	30	25	39.9857
27	100	40	30	25	29.9541
30	20	10	10	75	14.9516
20	100	10	10	75	14.6409
8	20	40	10	75	46.4029
26	100	40	10	75	30.6877
22	20	10	30	75	6.04177
13	100	10	30	75	9.93989
10	20	40	30	75	58.3225
25	100	40	30	75	46.2557
1	20	25	20	50	31.1004
6	100	25	20	50	26.2608
3	60	10	20	50	33.1223
12	60	40	20	50	56.3627
17	60	25	10	50	28.5023
19	60	25	30	50	29.5721
15	60	25	20	25	22.4834
29	60	25	20	75	25.512
11	60	25	20	50	32.4962
5	60	25	20	50	29.1136
28	60	25	20	50	30.54
9	60	25	20	50	31.2532
2	60	25	20	50	33.189
21	60	25	20	50	32.5003

The response surface for extraction of total phenolic compounds is given in Figure 1, showing the most significant interaction between input extraction variables. The significance of the effects of the independent variables and their influence on the dependent variables was checked using ANOVA analysis.

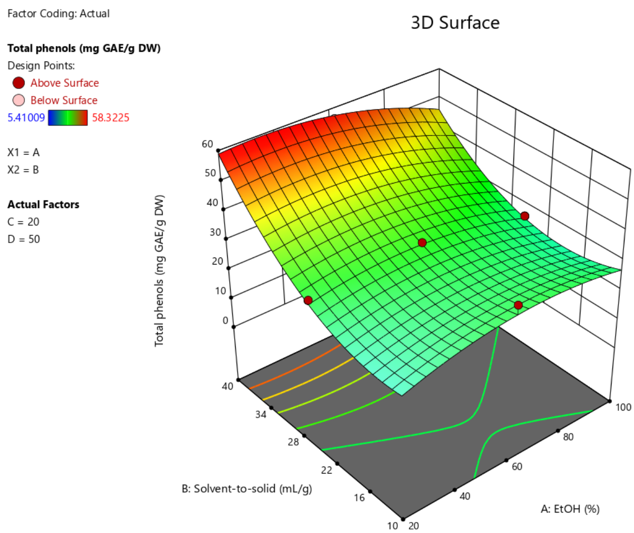

Figure 1. Response surface for extraction of total phenolic compounds; significant interaction between extraction variables

The variables with the highest influence on the extraction efficiency were solvent-to-solid ratio and temperature of extraction (highly significant, $p < 0.0001$), but also interaction between extraction variables solid-to-solvent ratio and ethanol composition ($p < 0.0001$). The model F-value of 53.42 implies the model is significant. The predicted R^2 of 0.8832 is in reasonable agreement with the adjusted R^2 of 0.9620; i.e. the difference is less than 0.2. Adequate precision measures the signal to noise ratio and obtained ratio of 28.181 indicates an adequate signal, along with a coefficient of variation of 9.57%.

Conclusion

In this work, the conditions of ultrasonic-assisted extraction of phenolic compounds from the *Salvia verticillata* L. root were simultaneously optimized using statistical tools - central composite design and Deringer's function, which enable detail insight into the extraction process. The proposed method for total phenolics extraction is fast, simple, low-temperature and does not involve large amounts of harmful chemicals. Obtained optimal conditions could be applied for further investigation of the biological activities of *Salvia verticillata* L. and related plant species.

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PHENOLIC CONTENT AND ANTIOXIDANT PROPERTIES OF SOME NATURAL COSMETIC CREAMS WITH PLANT-DERIVED INGREDIENTS AVAILABLE ON THE SERBIAN MARKET

Nevena Mihailović[1], Vladimir Mihailović[1], Nikola Srećković[1]

Abstract: The main goal of this study is to determine some properties of natural cosmetic creams that may have benefits for the skin. Five different cosmetic cream formulations with ingredients of botanical origin such as plant extracts, plant oil macerates, or essential oils were subjected to determine their phenolic content and antioxidant activity using the DPPH method. Total phenolic compounds were detected in all tested samples in the range of 1.7 to 0.2 mg GA/g but some samples did not contain flavonoids and did not possess antioxidant activity. The highest phenolic content and the strongest antioxidant activity were recorded for samples with the highest number of ingredients of plant origin.

Keywords: natural cosmetics, plant extracts, phenolic compounds, antioxidant activity

Introduction

The global cosmetics market value is expected to reach $716.6 billion by 2025. With such a considerable rise in the cosmetics market, many consumers seek products free or with minimal quantities of synthetic chemical compounds, considering such products as "greener" or "safer" for use and more beneficial for their healthy lifestyle. The development of greener and value-added cosmetics may emphasize the environmental, social, and economic positive impact of the cosmetic industry. These requirements have resulted in new approaches and innovations in the cosmetic industry, and, as a response, many new products are developed with ingredients of natural origin mainly derived from plants (Ferreira et al., 2022; Halla et al., 2018). One of the largest cosmetic product groups on the market is cream. The most common materials used in cosmetic cream preparation are water, oily materials (oils, fats), humectants, antioxidants, preservatives, pharmaceutical agents, and fragrances. Plant-derived ingredients in cosmetics are preferred instead of some synthetic

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compounds, mainly due to their better biocompatibility and biodegradability, low toxicity, and good protecting properties of the skin against UV irradiation, inflammation, infection, and hydration. According to the recent statistics natural cosmetics account for 3% of all cosmetic sales in Europe and their market share increased by 20% a year. The most common botanical cosmetic ingredients are vegetable oils, plant extracts, and essential oils (Dănilă et al., 2019). Many bioactive compounds presented in these plant-derivated ingredients, namely, phenolic acids, flavonols, anthocyanins, carotenoids, and vitamins, when isolated or in combined extracts have beneficial effects on the skin such as antioxidant, anti-inflammatory, anticarcinogenic, and antibacterial properties (Alaya et al., 2021).

There are many cream formulations on the Serbian market with the addition of some ingredients from botanical origin or declared as natural cosmetics. Most of them are declared as creams with specific botanical ingredients for skin care but there is no data about some specific biological effect of that formulation, there is only information about the biological effects of the herbal ingredient itself. The main goal of this study is to determine some properties of natural cosmetic creams available on the Serbian market that may have benefits for the skin. Five different cosmetic cream formulations with ingredients of botanical origin such as plant extracts, plant oil macerates, or essential oils were subjected to determine their phenolic content and antioxidant activity using the DPPH method.

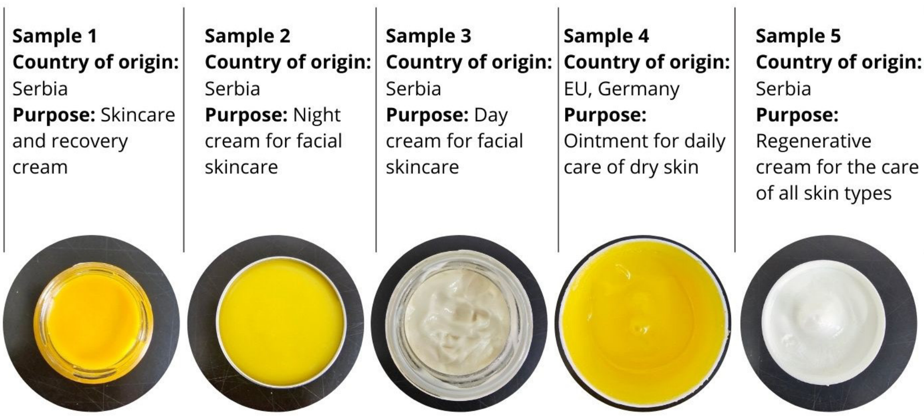

Figure 1. Photographs with main data of tested cream samples

Materials and methods

Extraction

Five different cosmetic cream formulations were collected in retail stores in Kragujevac (Serbia) during January 2024. The appearance, main data, and list of ingredients of analyzed samples are shown in Figure 1 and Table 1. For the determination of the total phenolic and flavonoid content of collected samples, as well as their antioxidant properties, creams were extracted with 96% ethanol. For this purpose, 5 g of cream sample was mixed with 50 mL of ethanol and heated with constant stirring in a water bath until completely melted. Then, the extraction was continued on an ultrasound bath (40°C) for 20 min. After cooling to room temperature, samples were left overnight in the refrigerator and then centrifuged for 15 min at 4000 rpm. The obtained ethanolic extract was decanted and used for further analyses.

Table 1. Ingredient list of tested cream samples

Sample	Ingredients (INCI)
1	Olea Europaea Fruit Oil, Calendula Officinalis Flower Oil, Aqua, Beeswax, Cocos Nucifera Oil, Hypericum Perforatum Oil, Lavandula Angustifolia Essential Oil, Calendula Officinalis Flower Extract, Tocopheryl Acetate, Propolis, Mentha Piperita Essential Oil, Hypericum Perforatum Flower Extract, Benzyl Alcohol, Dehydroacetic Acid
2	Olea Europaea Fruit Oil, Helichrysum Arenarium Flower Oil, Matricaria Chamomilla Flower Oil, Calendula Officinalis Flower Oil, Butyrospermum Parkii Butter, Beeswax, Aqua, Prunus Dulcis Oil, Macadamia Ternifolia Oil, Corylus Avellana Oil, Persea Gratissima Oil, Rosmarinus Officinalis Essential Oil, Tocopheryl Acetate, Melaleuca Leaf Essential Oil, Benzyl Alcohol, Dehydroacetic Acid
3	Aqua, Helichrysum Angustifolium Flower Water, Sorbitan olivate (and) Cetearyl olivate, Glycerin, Niacinamid, Simmondsia Chinensis Oil, Butyrospermum Parkii Butter, Cannabis Sativa Seed Oil, Olea Europaea Fruit Oil, Calendula Officinalis Flower Oil, Squalane, Aloe Barbadensis Leaf Extract, Tocopheryl Acetate, Cananga Odorata Flower Essential Oil, Benzyl Alcohol, Hyaluronic Acid, Cucumis Sativus Fruit Extract, Calendula Officinalis Flower Extract, Rubus Idaeus Seed Extract, Sodium gluconate, Dehydroacetic Acid
4	Petrolatum, Paraffinum Liquidum, Helianthus Annuus Seed Oil, Glycine Soja Oil, Parfum, Calendula Officinalis Flower Extract, Tocopherol, Daucus Carota Sativa Root Extract, Beta-Carotene, Benzyl Alcohol
5	Aqua, Stearic Acid, Calendula Officinalis Flower Extract, Octyldodecanol, Cetyl alcohol, Glycerin, Caprylic/Capric Triglyceride, Triethanolamine, Phenoxyethanol, Ethylhexylglycerin, Tocopheryl Acetate, Parfume, Tetrasodium EDTA, Rosmarinus Officinalis Leaf Extract

Determination of total phenolic and flavonoid content

Total phenolic content in cosmetic creams was determined in their ethanolic extracts using Folin-Ciocalte reagent and the result is expressed in gallic acid equivalents (mg GA/g of cream). The total amount of flavonoids was determined using $AlCl_3$ as regent by spectrophotometric method and expressed in quercetin equivalents (mg Qv/g cream). The detailed procedures are described in a previously published paper by Srećković et al. (2020).

Determination of antioxidant activity using the DPPH method

The same volumes of ethanolic extract of cream samples and DPPH solution (80 μg/mL) prepared in ethanol were mixed to determine their potential to neutralize DPPH radicals. The samples were incubated for 30 min at room temperature in a dark place and the absorbance of the samples was recorded at 517 nm (Kumarasamy et al., 2007). The antioxidant activity of cream samples was expressed in Trolox equivalents per gram of cream (mg Trolox/g of cream).

Results and discussion

Table 2. Organoleptic characteristics of cream samples

Sample	Appearance	Color	Consistency	Shine
1	homogeneous ointment	orange	semi-solid	slightly shiny
2	homogeneous ointment	dark yellow	semi-solid	slightly shiny
3	homogeneous emulsion	cream white	semi-solid	pearlescent
4	homogeneous ointment	orange	semi-solid	shiny
5	homogeneous emulsion	white	semi-solid	pearlescent

The five different cosmetic creams (Figure 1) that contained ingredients from plant origin were collected in local stores in Kragujevac to determine their antioxidant potential and phenolic content. These properties of cosmetic formulations mainly originate from their botanical ingredients, which are significant for the biological activity of cosmetic formulation and their beneficial effects on the skin. All analyzed samples were semi-solid consistency, homogeneous ointments, or emulsions, and yellow, orange, or white color (Table 2). All samples contained a minimum of two ingredients from plants and calendula flower extract (*Calendula officinalis* L.) (Table 1). According to the ingredients listed on the cream packaging presented in Table 1, samples 1, 2, and 3 are mainly composed of plant oily macerates, plant oils, extracts end essential oils, while the main ingredients of samples 4 and 5 were synthetic components such as perfumes and hydrocarbon derivatives of petroleum origin.

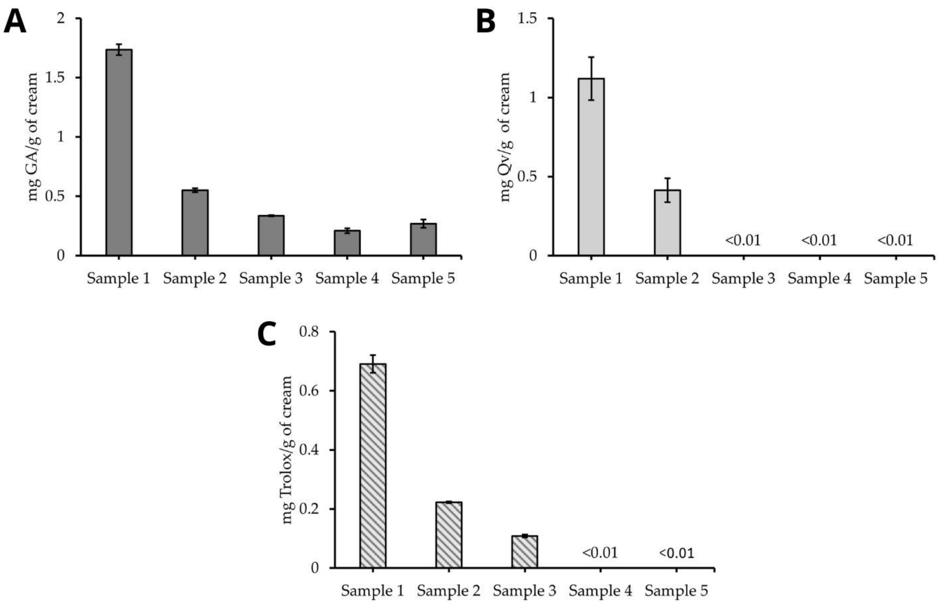

Figure 2. Total phenolic content (A), total flavonoid content (B), and antioxidant activity (C) of analyzed cosmetic creams

The total phenolics were quantified in all cream samples, while detectable amounts of flavonoids were found only in samples 1 and 2 (Figures 2A and 2B). Also, both samples 1 and 2, followed by sample 3 contained the highest total phenolic content and displayed antioxidant activity determined using the DPPH method. These results indicate a correlation between total phenolic and flavonoid content in creams with their antioxidant activity. The significantly higher total phenolic and flavonoid content, as well as antioxidant activity in comparison with other samples showed sample 1. This sample, according to its ingredients list, contains all natural ingredients (apart from low quantities of benzyl alcohol and dehydroacetic acid as preservatives) with high content of *C. officinalis* macerate in olive oil, as well as *C. officinalis*, *H. perforatum*, and propolis extracts. Significant phenolic and flavonoid contents and antioxidant activity were recorded for sample 2 rich in plant oils and different plant oily macerates.

Conclusion

These results indicate that cosmetic formulations that contain plant-derived ingredients do not always exert beneficial effects (such as antioxidant activity) on users, just because they contain some bioactive plant ingredients. It is obvious that there must be a sufficient amount of selected plant-derived ingredients in any formulation in order to exert some biological activity.

Acknowledgment

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INFLUENCE OF CONTROLLED RADIATION ON THE PROPERTIES OF BIOCOMPATIBLE SILICONE ELASTOMERS

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Abstract: For everyone involved in the synthesis, processing and application of elastomers, it is necessary to know how an elastomeric material will behave under the influence of different types of energy. The aim of this work is to establish the resistance of biocompatible silicone elastomers to the permitted doses of radiation. Elastomeric materials were irradiated with gamma rays in a Co-60 radiation chamber with a dose of 25 kGy/h. By determining the various properties before and after irradiation of the silicone material, it was found that the resistance to radiation is significantly improved in samples reinforced with silicon (IV) oxide nanoparticles. FTIR spectroscopy was used to confirm the assumed mechanism of the crosslinking reaction of siloxane. The analysis of the thermal properties of the synthesized siloxane materials was performed using a DSC device, which showed that the proportion of nanofillers and vinyl functional groups significantly affects the values of the melting temperatures before and after irradiation.

Keywords: elastomers, nanocomposites, network, polymer siloxanes, thermal properties, radiation.

Introduction

In the middle of the last century, experiments began with the use of ionizing radiation in order to initiate the polymerization reaction, but also additional crosslinking, polymerization when graft copolymers are formed, as well as modifications of the synthesized polymer. The mechanical properties of the polymer are significantly changed by high radiation energy. These may be the results of either cross-linking or degradation of the polymer chains.

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Properties such as tensile strength, hardness, modulus and elongation at break are reduced as a result of chain separation, while cross-linking has the opposite effect on these properties. During elastomers aging there is possibility of network chain degradation or disintegration, internetworking and changes in chemical structure of some network chain parts. Large changes in physical and chemical properties can be observed when elastomeric materials are exposed to high temperatures and/or air and radiation. These changes can be divided into three typical forms: post-crosslinking, which results in a higher crosslinking density and an increase in the brittleness of the elastomer; breaking of the chains, which leads to a decrease in the length of the chain and thus the average molecular weight, so the material becomes softer, and a chemical change in the polymer chain with the formation of polar or other groups. These researches are still very relevant today, as can be concluded from the literature (Bhattacharya, 2000), (Davenas et al., 2002), (Ivanov, 1992), (Woods and Pikaev, 1994). In biomedicine, the advantages of radiation techniques are particularly significant because they enable biostability, biocompatibility and exceptional purity of the product, which can be sterilized at the same time. The latest research is related to the application of ionizing radiation to the preparation and processing of nanoparticles as well as nanocomposites (Ren et al., 2003), (Liu et al., 2003), (Kickelbick, 2003), (Zhao et al., 2003). Siloxanes are widely used in medicine owing to their high biocompatibility, biological inertness, non-toxicity (they do not cause irritation), efficiency (characterized by their ability to be applied to the skin and to be permeable to therapeutic substances) and stability (high chemical inertness and ability to retain physical and chemical properties at the skin’s temperature), (Sanchez and Ribol, 1994). The crosslinking process most commonly used nowadays in polysiloxane chains is known as “addition reaction” (Figure 1). Since functional silicone polymers are becoming more and more available, addition reactions in silicone coatings have now started to develop (Kanar, 2006), (Okumara and Ito, 2001).

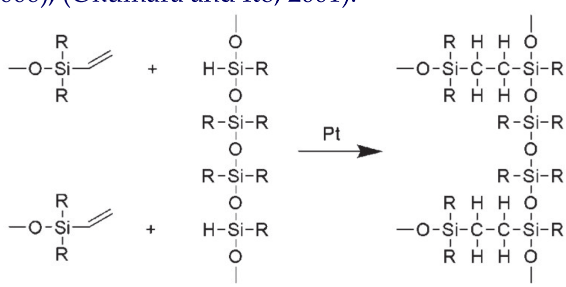

Figure 1. Addition crosslinking of siloxane by hydrogen siliconization using a Pt - catalyzed

In this work, the structure of the siloxane matrix before and after irradiation was studied. For this purpose, polymer networks with α,ω-divinyl poly(dimethylsiloxane) and poly(methyl-hydrogensiloxane) precursors were synthesized. Hydrophobic nanosilicon(IV)-oxide particles from the master batch were used as fillers for the preparation of nanocomposites and vinyl groups for radiation resistance.

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 35/65). 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. Pre-formulated concentrate "masterbatch" (MB) was also used as a reactive component to improve the properties of siloxane elastomers. The masterbatch used is a highly filled siloxane mixture containing vinyl functional groups and reinforcing fillers. The applied masterbatch contains 3.8 wt% silicon(IV)-oxide, with a hydrophobic surface, an average particle size of 1 μm and an active surface of 105 m^2 g^{-1}. 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. It can be also seen in Figure 2 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 fillers. The sample with excess vinyl siloxane shows a peak of weaker intensity at 3080 cm^{-1}, arising from an unreacted double bond. In the case of the ratio H/Vi, the addition of the masterbatch increases the concentration of unreacted vinyls, which in this case is also observed in the spectra as a slight increase in the band at 3080 cm^{-1}.

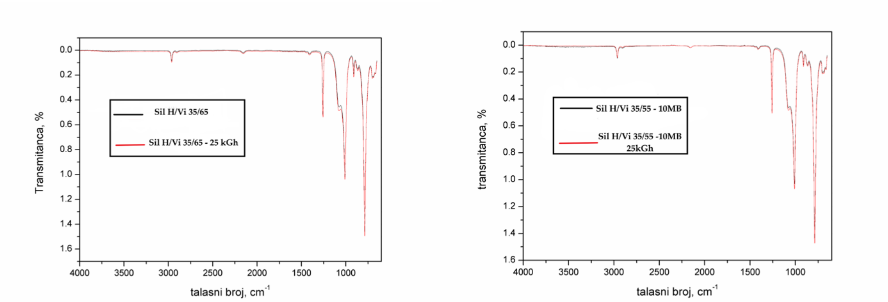

Figure 2. FTIR spectra of siloxane elastomers: hydrogen/vinyl siloxane elastomers nanocomposites (ratio Sil H/Vi 35/65; ratio Sil H/Vi 35/55 - 10MB) irradiated with 25 kGy/h

Table 1. Mechanical properties of prepared siloxane elastomers and their nanocomposites

	Tensile strength, MPa	Elongation at break, %	Shore A Hardness
Sil H/Vi 35/65	2.09	19.95	38
Sil H/Vi 35/55-10 MB	0.401	46.2	45
Sil H/Vi 35/65 (25 kGy/h)	2.18	19.01	41
Sil H/Vi 35/55-10 MB (25 kGy/h)	0.780	40.33	46

The addition of masterbatch reduces the breaking strength, which is especially pronounced when adding 10 wt% MB, because the structure of the already insufficiently cross-linked siloxane is disturbed and larger fractals appear in the network, so the strength of the network itself is lower and the deformation energy is reduced. It can be concluded that the addition of masterbatch leads to the increase in elongation at break and increases hardness of composites, which can be explained by the lower degree of interactions of the hydrophobic siloxane matrix with a hydrophobic filler. Radiation leads to additional cross-linking and increases the values of tensile strength and hardness and reduces elongation. With the addition of MB and nanoparticles, that effect is more pronounced.

The increase in the MASTERBACH loading significantly affected thermal properties of siloxane elastomers. In pure silicon elastomers (ratio Sil H/Vi 35/65) melting temperatures (Tm) were about -55 °C, but with addition of the nanofiller, Tm values changed. (Fig. 3). At the H/Vi ratio of 35/65, the addition of MB leads to an additional decrease in the Tm value due to the effect of plasticization, because the additional MB has nothing to react with, so vinyl polysiloxanes remain in a large excess in the system, Figure 3. However, this leads to more intense phase separation, so there is an increase in the value of the melting enthalpy, because the cross-linked domains are now more easily oriented.

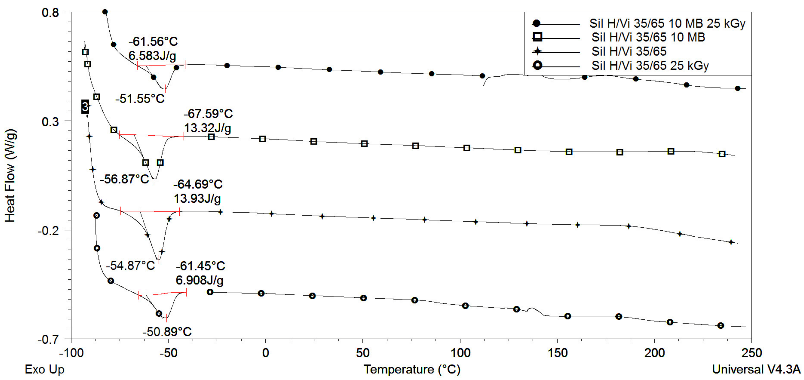

Figure 3. DSC thermograms of the silicon elastomers and their nanocomposites with hydrophobic fillers

In the case of irradiated samples, there is a decrease in the value of the melting enthalpy, which can be interpreted as the fact that the radiation causes disordering of polysiloxane chains, that is, there is an increase in the value of the melting temperature, which can be explained as additional cross-linking, that is, a decrease in the mobility of the siloxane chains.

Conclusion

Polysiloxanes are certainly one of the most interesting class of polymers. In this paper, the influence of radiation 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. 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 master bach increased Tm values. The obtained results indicated that the resistance to radiation has been attained in different properties of silicon(IV)-oxide nanoparticles reinforced samples with respect to unloaded ones.

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GERANIUM MACRORRHIZUM, THE CURE IN YOUR GARDEN. NEW METHOD OF GERMACRONE ISOLATION

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Abstract: *Geranium macrorrhizum* L., commonly known as "*Zdravac*" is an herbaceous medicinal plant that is traditionally used to treat skin diseases such as cuts, burns, and eczema. The essential oil obtained from the aerial parts of the plant is widely used in ethnopharmacology, perfumery and cosmetics. Germacrone is a bioactive natural compound found as part of the essential oils of different plants that are widely distributed throughout the world. This multifaceted chemical entity has become a point of focus during recent years due to its numerous pharmacological applications. The aim of the present study was to determine the chemical composition of the essential oil obtained from aerial parts of *Geranium macrorrhizum* and to develop the new method of pure germacrone isolation as its main component.

Keywords: *Geranium macrorrhizum*, essential oil, germacrone, isolation

Introduction

Geranium macrorrhizum L. occurs in the Southern Alps, Apennine and Carpathian Mountains, extending to southern Greece in the Balkan Peninsula. In Europe it is increasingly cultivated on account of its ornamental flowers. Being winter-hardy it naturalizes easily in parts of central Europe. *G. macrorrhizum* is highly valued in ethnopharmacology of the Balkan region, for its aromatic essential oil, and it is known in Serbia as wild geranium or "*Zdravac*", with reference to its Serbian name zdravats, meaning "health". It possesses a wide range of beneficial properties such as antimicrobial, hypotensive, spasmolytic, astringent, cardiotonic, antioxidant, and sedative activities.

Germacrone is a sesquiterpene which is found as part of the essential oils of different plants that are widely distributed throughout the world. Among them, several species of the genus *Dracocephalum* (Ahmadi and Mirza, 2001), *Curcuma* (Srivastava et al., 2006), *Rhododendron* (Doss et al., 1986), *Zingiber* (Bordoloi et

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al., 1999) etc. In addition, germacrone is noteworthy for its abundance in different essential oils (with yields higher than 20% of the total oil). Among them is the essential oil obtained from the aerial parts of *G. macrorrhizum* with a germacrone content of over 50% as the major component (Chalchat et al., 2002). As a result, it is possible to separate germacrone from the essential oil that contains it by a simple crystallization, favoring its use at the industrial level.

Therefore germacrone has become a point of focus during recent years due to its numerous pharmacological applications, e.g., anticancer, anti-inflammatory, antiviral, antioxidant, anti-adipogenic, anti-androgenic, antimicrobial (Carlton et al., 1992), insecticidal (Doss et al., 1980), and neuroprotective (Yamazaki et al., 1989). It is also an effective inducer of cell cycle arrest and apoptosis in various cancers (breast, brain, liver, skin, prostate, gastric, and esophageal) via modulation of different cell signaling molecules (Morikawa et al., 2002) and pathways involved in cancer proliferation (Ogunwande et al., 2005). So, the reported data collected from various shreds of evidences recommend that this multifaceted compound could serve as a potential drug candidate in the near future.

The aim of the present study was to determine the chemical composition of the essential oil obtained from aerial parts of *Geranium macrorrhizum* and to develop the new method of pure germacrone isolation (HPLC grade) as its main component. For this purpose, preparative thin layer chromatography (preparative TLC) was used as a fast, inexpensive and useful technique for the purification of small quantities of sample. It permits a rapid separation and isolation/recovery of components in a mixture.

Materials and methods

The plant was harvested at full flowering stage (June 2019) on hill near the town of Despotovac, (Serbia). The essential oil was isolated from fresh aerial parts by Clevenger type hydrodistillation and analyzed by a 7890/7000B GC/MS/MS triple quadrupole system in MS1 scan mode (Agilent Technologies, USA) and equipped with a fused-silica capillary column Agilent HP-5 MS (30 m × 0.25 mm, 0.25 μm). Analysis was carried out in the following conditions: He as carrier gas at a flow rate of 1.0 mL/min, GC oven temperature was kept at 45 °C for 2.25 min and programmed to 290 °C at a rate of 4 °C/min, split ratio was adjusted at 40:1, injection volume 1 μL. The injector temperature was set at 230 °C. Ionization mode was electronic impact at 70 eV. A purity check of the isolated germacrone was performed under the same conditions.

Isolation of the germacrone from the essential oil was carried out on the preparative Supelco TLC plate (200 x 200 mm, layer thickness 1 mm, particle size 20-40 µm), glass support silica gel 60 matrix with fluorescent indicator. The elution system consisted of a mixture of hexane : ethyl acetate / 8 : 2. The spots with the silica (visible on 354 nm UV lamp) were scraped, extracted with hexane and identified by the GC/MS.

HPLC analysis was performed on Agilent 1200 HPLC chromatograph with DAD detector and analytical ZORBAX Eclipse XDB C18 column (4.6 x 50 mm, 5 µm). The purity of the isolated germacrone was checked on the above mentioned chromatograph with solvent system consisting of methanol : water (1% HCOOH) / 8 : 2, . isocratic elution, flow rate 0.5 mL/min and with an injected sample volume of 5 µL, concentration of 5 mg/mL.

The solvents and other chemicals used for the analysis were of HPLC grades purchased from J.T. Baker.

Results and discussion

In a essential oil of the *G. macrorrhizum* essential oil, 89 components were identified in total, what makes a little bit over 93% of present compounds. The most dominant class of compounds was found to be sesquiterpenoids with the share of 87.2%. Germacrone was identified as the major constituent representing 54.2% of the essential oil obtained from the plant in the full flowering stage. GC/MS chromatogram of the investigated sample is shown on Figure 1.

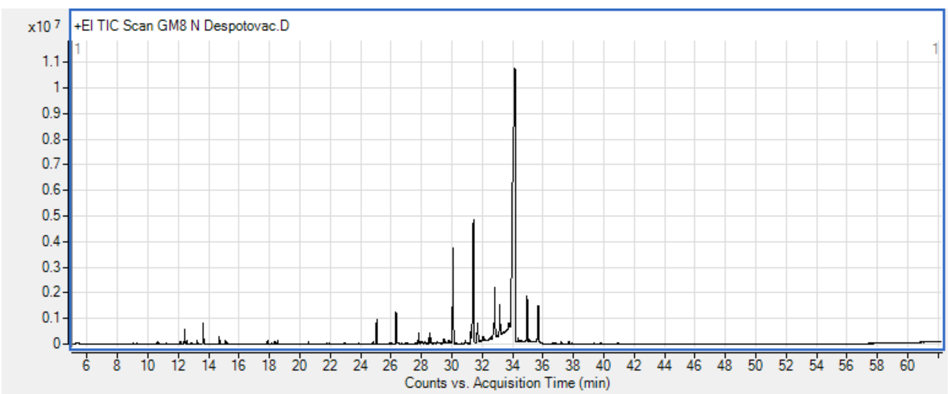

Figure 1: GC/MS chromatogram of *G. macrorrhizum* essential oil

The amount of applicated essential oil was 200 mg and the yield of isolated germacrone by the preparative TLC technique was 94.4 mg (47.2% of the mass of crude essential oil sample).

According to the GC/MS analysis of the investigated TLC spot, the purity of the germacrone was not satisfactory since it was 87.6%, nor was the quality of the separation since an β-elemenone signal, with a share of 5.7%, was also observed (Figure 2).

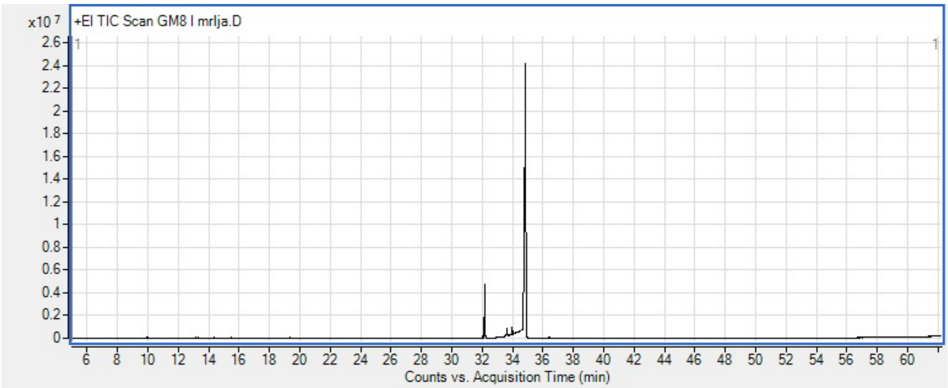

Figure 2: GC/MS chromatogram of the germacrone TLC spot

The rearrangement of germacrone to β-elemenone (Scheme 1) is common during gas chromatographic (GC) analysis based on its pyrolitic transformation (Cope sigmatropic rearrangement). The conversion taking place in the injector port at temperatures above 250°C as well as on the column. While production of germacrone in the injector is evident from the gas chromatographic trace, β-elemenone produced from germacrone during its migration down the column is difficult to detect because of the diffuse nature of the chromatographic peak produced by this process (peak broadening). So, it is clear that this process poses problems for quantifying germacrone by this method and casts doubt on the authenticity of reports.

Scheme 1: Thermal rearrangement of germacrone to β-elemenone.

Therefore, the best way to overcome such problems is quantification using the HPLC method. The HPLC chromatogram of the isolated germacrone is shown in the Figure 3.

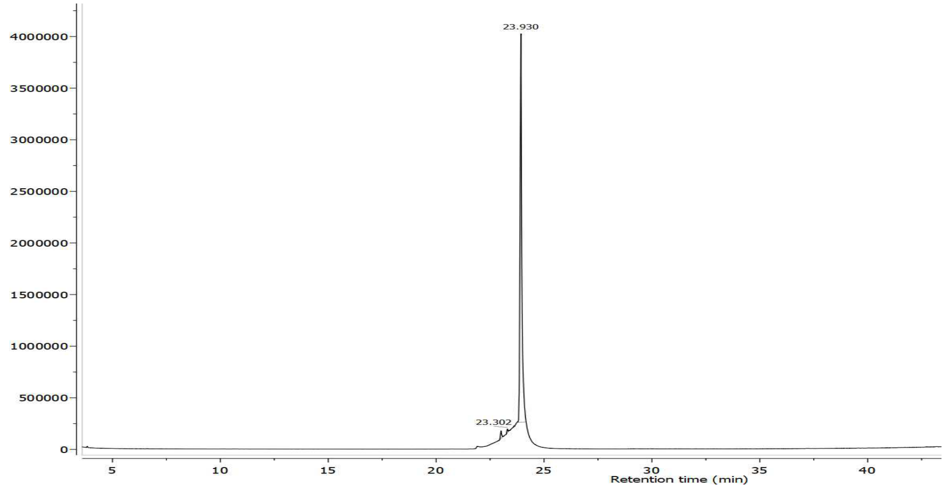

Figure 3: HPLC chromatogram of the isolated germacrone

HPLC analysis of the extracted spot revealed the presence of germacrone but no detectable β-elemenone, implying that β-elemenone detected by GC is entirely an artifact. The sharpness of the β-elemenone GC/MS peak suggested that it was generated in the injection port (high temperature and short residence time). The purity of isolated germacrone, according the HPLC method is 99.1% which is far above the limit for HPLC standards (>98%).

Conclusion

Although there are many techniques for isolating the components of complex mixtures such as essential oils, all of them have some disadvantages. Some of them are too complex such as High-speed counter-current chromatography (HSCCC), some require the use of expensive equipment giving low yields like preparative HPLC, some are too slow (column chromatography) and some of them do not allow the isolation of components of satisfactory purity such as flash chromatography. The obtained results show that preparative TLC chromatography can be used to isolate germacrone, in relatively large quantities, in a fast and inexpensive way with high yield and satisfactory purity, without any preliminary purification. In this way, it is possible to apply germacrone (in a large scale) in pharmacy, laboratory practice or even in industry.

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FATTY ACIDS CONTENT IN WATERMELON, MELON, SQUASH AND ZUCCHINI SEEDS

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Abstract: Seed samples of ten zucchini, melon, squash and watermelon samples were examined by gas chromatography-mass spectrometry (GC-MS) analysis for the presence of fatty acids. Fatty acid composition was significantly different among various samples. Hexadecanoic acid was found in all analyzed seed samples, whereas 9,12,15-octadecanoic acid was found only in zucchini seed samples. Unsaturated fatty acids, which possess multiple health benefits, were present in all samples, in various amounts. The high concentration of essential unsaturated fatty acids in analyzed seed samples indicates that they could be used as a source of these valuable compounds in human nutrition.

Keywords: fatty acids, seed, zucchini, melon, watermelon, squash

Introduction

Oils and fats in food are composed of four different types of fatty acids: (FAs) – polyunsaturated, (PUFAs), monounsaturated - (MUFAs), saturated - (SFAs) and trans fatty acids - (TFAs). (Lehotay and Hajšlová, 2002) Fatty acids can regulate lipid metabolism at three different levels: by interaction with enzymes to affect their activity, by interaction with nuclear transcription factors to modulate gene expression and affect mRNA stability and thus regulate expression of enzymes. (Yilmaz et al., 2013) It is recommended to increase the intake of PUFAs and to decrease the intake of SFAs and TFAs, because TFAs affect cholesterol levels in much the same ways as saturated and trans fats increase your risk of developing coronary artery and heart diseases. (Backholer and Peeters, 2012)

Watermelon, melon, squah and zucchini are commonly consumed, and eaten all around the world. They contain significant quantities of seeds that have been discarded. These seeds can contain high functional and nutritional

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potential compounds. Also, seeds of mentioned fruits and vegetables were found to be potential sources of oil for biodiesel production. (Abishri et al., 2013)

The aim of this study was to examine the fatty acid content of seed samples from zucchini (3 samples), watermelon (3 samples), melon (1 sample) and squash (3 samples).

Materials and methods

Chemicals and samples

Chloroform, methanol, hexane and boron trifluoride 10% methanolic solution were purchased from Sigma Aldrich, anhydrous magnesium sulhate and potassium hydroxide were obtained from Merck.

Samples were purchased in the local market in Niš. Flesh was separated from seed, and seeds were homogenized in mortar.

Extracts preparation

2g of each seed sample was extracted with chloroform/methanol mixture for 10 min, at 40 °C. The resulting mixtures were evaporated to dryness and dissolved in 5 mL of methanol. Extracted samples were hydrolyzed with 1 mL of methanolic KOH solution and then a complete derivation was done with 1 mL of boron trifluoride in methanol. The methyl esters were extracted with hexane, and evaporated to dryness. Dry residue of methyl esters was dissolved with hexane prior GC MS analysis.

GC MS analyisis

All samples were analyzed on a 7890/7000B GC–QqQ-MS system (Agilent Technologies, USA). Chromatographic separations were conducted using a HP-5MS (5% phenyl methyl siloxane) column (30 m x 250 mm x 0.25 mm). The GC oven was operated with the following temperature program: the column temperature was 70 °C, for a 2.25 min hold, and then programmed to increase to 300 °C at a rate of 5 °C/min and then held for 10 min. The splitratio was 1:20, and the injected volume was 1 μL. Helium was used as carrier gas with a flow of 24 mL min^{-1}. MS conditions were as follows: an ionization voltage of 70 eV, acquisition mass range of 40–560, and a scan time of 0.32 s.

Results and discussion

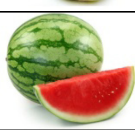
Gas chromatography–mass spectrometry analysis (GC–MS) is a powerful tool for qualitative and quantitative analysis of various compounds present in natural products, and the technique has been widely applied in medical, biological and food research. (Kaluzna Czaplinska, 2007) The amount (%) of fatty acids in analyzed seed samples was presented in Table 1. Fatty acid composition was significantly different among various samples. Hexadecanoic acid was found in all analyzed seed samples, in high amounts. The highest amount of this acid was found in mini watermelon seed sample (49.37%) and green round squash (49.11%). Various bioactivitie properties of watermelon seed oil have been reported including antioxidant, anti-inflammatory, cardioprotective and antimicrobial activities. (Petchsomrit et al., 2020) The content of saturated fatty acids in mini watermelon seed samples was twice higher than unsaturated acids in this sample. Similar content of hexadecenoic acid as in mini watermelon and green round squash was found in other two analyzed zucchini samples (48.35 and 45.69%). Twice lower content of this fatty acid was found in squash and melon seed samples.

9,12-octadecanoic acid was also found in all analyzed seed samples. The highest content of this acid was reported in *Cucurbita maxima* roter zentner seed (45.62%), which is the most abundant fatty acid in this sample. Seeds of species belong to *Curcubitacea* family, which are considered as waste, can be rich source of bioactive compounds. Siano et al. (2016) found that saturated fatty acids and monounsaturated fatty acids of *C. maxima* produced in Italy showed similar values (25.20% and 25.54%, respectively), while the polyunsaturated fatty acids content was 48.14%, which is in agreement with the results found in this study.

However, 9-octadecanoic acid was found in squash and melon seed samples, but not in zucchini seed samples. Gomes et al. (2020) analyzed winter squash seed samples and found that unsaturated fatty acids are 70% of fatty acids in seed samples, with 9-octadecanoic as leading compound, which is in agreement with results of this study. Unsaturated fatty acids should be consumed more often since this reduces the risk of cardiovascular diseases. The highest content of 9-octadecanoic acid was found in *Cucurbita pepo* subsp. Pepo (47.48%), whereas the lowest was in orange heart watermelon seed samples (11.47%).

9,12,15-octadecanoic acid was found only in zucchini seed samples (18.98-34.22%), whereas in other seed samples was below limit of detection. This acid is widespread in plants tissues. Significant amount of 9,12,15-octadecanoic acid was found in the lipids of chloroplast membranes. (Plemenkov 2001)

Table 1. **Fatty acids content in analyzed seed samples**

Sample		Fatty acid (%)				
		Hexadec. acid	9,12-Octadec. acid	9-Octadec. acid	9,12,15-Octadec. acid	Octadec. acid
Cucurbita pepo crone (Ten commandments gourd)		22.39	37.53	36.41	n.d.	3.68
Cucurbita maxima roter zentner		20.3	45.62	27.75	n.d.	6.33
Cucurbita pepo subsp. pepo		21.92	23.71	47.48	n.d.	6.89
Sweet gourmet zuchinni		48.35	8.62	n.d.	34.22	5.45
Green round squash		49.11	14.34	n.d.	23.11	13.43
Light green round squash		45.69	24.45	n.d.	18.98	7.79
Yellow heart watermelon		35.77	38.81	11.81	n.d.	13.62
Orange heart watermelon		41.28	33.46	11.47	n.d.	13.79
Mini watermelon		49.37	23.88	13.47	n.d.	13.28
Melon		29.66	38.33	21.65	n.d.	10.36

n.d. – not detected

Conclusion

This study has shown high variation of fatty acids in seeds from melon, watermelon, zucchini and squash. Our results showed that zuchinni seed was rich in 9,12,15-octadecanoic acid, which was not found in other analyzed samples. On the other hand 9-octadecanoic acid was not found in zucchini seed samples, but it was found in other analyzed samples. Fatty acid profile is species depending and could serve as a marker for species determination and confirmation.

Acknowledgement

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UPTAKE OF HEAVY METALS BY WHITE CLOVER (*TRIFOLIUM REPENS* L.) – POT EXPERIMENT

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Abstract: This study investigated the uptake of some heavy metals (Cd, Cr, Cu, Ni, and Pb) by white clover (*Trifolium repens* L.) using a pot experiment. Plant and soil samples were prepared through wet digestion and analyzed by ICP-OES. The obtained results were discussed in terms of metal concentrations, translocation factors (TF), and enrichment factors (EF). Metal concentrations were higher in the roots compared to the shoots of the plant. White clover growing in Cd-contaminated soil absorbed significant amounts of Cd in both the roots and aboveground parts.

Keywords: soil, white clover, heavy metals, pot experiment, ICP-OES

Introduction

The application of different fertilizers and other agrotechnical practices, in addition to improving yields of various crops, can lead to an increase in pollutant concentrations in the soil and plants. One of the most significant problems is soil pollution with heavy metals. Some of the anthropogenic sources of soil pollution with heavy metals include the use of fertilizers, pesticides, the use of wastewater for irrigation, mining, and industrial waste (Jing et al., 2018). Heavy metals present in the soil can be absorbed by plants grown in contaminated soil, leading to negative consequences for both the plants themselves and the animals and humans who consume the plants as food.

White clover (*Trifolium repens* L.) is a plant that belongs to the legume family. It is used in animal grazing and, when mixed with other grasses, serves as hay. White clover is a good source of protein and minerals, thereby improving the quality of animal products especially milk. In addition to its common use in various parts of the world, people also apply it in traditional medicine as a medicinal plant. For example, in Turkey, white clover is used as

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an antiseptic, analgesic, and sedative. In Iran, white clover is employed for skin ailments, with the flowers used as an analgesic (Ahmad and Zeb, 2020).

Since white clover is commonly used in animal nutrition, many researchers have investigated the uptake of heavy metals by this plant. Lanier et al., 2015, examined the influence of Pb and Cd soil pollution on the uptake of these metals using pot experiment. Increased concentrations of Pb and Cd in the soil led to an increased concentration of these metals in the plant. Simultaneous pollution of soil with Pb and Cd did not significantly affect the metal content compared to the content where the plant was grown in soil contaminated with only one metal. Memic et al., 2023, determined the concentrations of heavy metals in samples of white clover that grown on polluted and unpolluted soil in Bosnia and Herzegovina. The metal concentration in white clover depended on the sampling location and the time of year. The most abundant element in the root was Fe, while in the aboveground parts, Fe and Zn were the most abundant. Bidar et al., 2007, studied the content of Cd, Pb, and Zn in field samples of white clover – the concentrations of these metals were higher in the roots compared to the aboveground part of the plant.

The aim of this work is to examine the uptake of Cd, Cr, Cu, Ni, and Pb by white clover grown on soils artificial contaminated with those metals, both individually and in combination in a pot experiment.

Materials and methods

To establish a pot experiment, untreated soil that had not undergone agrotechnical practices for several years was utilized. The soil, obtained at a depth of approximately 20 cm, was air-dried for around two weeks and then sieved. White clover seeds (*Trifolium repens* L.; Semenacoop, Novi Sad) were purchased from an agricultural pharmacy.

For the model system units, 400 g of soil was measured and distributed into each of the seven pots. The amounts of heavy metals added were equivalent to the concentration found in the test soil except for Cd (28x higher). A 100 ml volume of the heavy metal solution was uniformly applied to treat all units of the model system. The first unit of the model system received treatment with deionized water only, serving as a reference unit. The second unit was treated with a mixture of all five tested heavy metals, while the following five units were individually treated with one metal each. After achieving suitable soil moisture levels after a few days, the clover was sown, with twenty seeds planted in each pot.

After 100 days of sowing, the plants were removed, and the roots were separated from the aboveground parts. All plant material was dried and prepared for ICP-OES analysis according to Bargagli et al., 2000. Also, soil samples, were dried and prepared using pseudo-total extraction method with aqua regia according to 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 analysis are presented in Table 1. Doping the soil with heavy metal solutions led to an increase in metal concentrations compared to the reference unit of the model system. Metal concentrations were highest in the model system unit where the soil was doped with a mixture of heavy metals.

Table 1. Heavy metals in soil (mean ± SD, mg kg^{-1} of dry matter)

Sample	Cd	Cr	Cu	Ni	Pb
Ref-S	3.65±0.02	19.98±0.08	18.20±0.04	25.65±0.03	19.51±0.03
Cd-S	36.67±0.05	21.02±0.07	19.54±0.02	26.6±0.2	20.6±0.2
Cr-S	4.040±0.005	26.9±0.3	20.3±0.4	26.8±0.2	20.97±0.08
Cu-S	3.753±0.003	20.1±0.3	24.1±0.3	26.0±0.2	19.52±0.07
Ni-S	3.86±0.02	19.78±0.09	18.1±0.2	30.9±0.2	20.12±0.07
Pb-S	3.70±0.02	19.0±0.3	18.1±0.3	24.77±0.03	94.4±0.5
Mix-S	55.0±0.5	34.9±0.4	49.8±0.6	43.9±0.4	94.7±0.4
LV*	0.8	100	36	35	85

*limit values of heavy metals in the soil

In the reference unit, except for Cd, metal concentrations were lower than the limit values (Regulation on limit values of polluting, harmful and dangerous materials in the soil, The Official Gazette of the Republic of Serbia, 2019). The concentrations of Cd in all model system units exceeded the limit value. Doping with heavy metals resulted in exceeding the limit values for Cu in the unit where the soil was doped with a metal mixture, for Pb in units where the soil was doped individually with Pb and with a mixture and for Ni in Mix-S sample.

The concentrations of heavy metals in the roots and shoots of white clover are provided in Table 2 and Table 3, respectively. The treatment with a mixture of heavy metals completely inhibited the growth of white clover, likely due to the synergistic toxic effect of the heavy metals. Doping the soil with heavy metal resulted in an increased content of heavy metal in the roots compared to the reference unit. The highest content of heavy metal was observed in the unit where the soil was treated with that particular metal, with two exceptions for Cr and Pb. Based on the results, it can be observed that an increase in the concentration of one metal in the soil led to a decrease the content of other analyzed metals in the roots of white clover compared to reference unit, where soil is dopped with Cr, Cu, Ni, and Pb. However, the increase in the concentration of Cd in the soil led to a increase in the concentrations of metals in the roots, except for Cu.

Table 2. Heavy metals in roots (mean ± SD, mg kg^{-1} of dry matter)

Sample	Cd	Cr	Cu	Ni	Pb
Ref-R	4.048±0.000	6.9±0.4	16±2	12.1±0.2	18±2
Cd-R	126.1±0.8	11.8±0.8	4.0±0.4	17±2	17.5±0.3
Cr-R	1.05±0.02	10.1±0.2	13.1±0.3	8.81±0.08	9.1±0.2
Cu-R	2.37±0.03	5.48±0.08	16.72±0.08	9.02±0.06	10.4±0.3
Ni-R	1.321±0.000	5.33±0.03	13.1±0.1	18.20±0.08	7.3±0.1
Pb-R	4.11±0.07	5.0±0.4	12.5±0.5	11.6±0.3	54.8±0.9

The highest concentrations among the analyzed elements in the shoots are observed for Cu, while the concentrations of Cd, Cr, Pb, Ni are much lower compared to Cu in most model systems. These concentration values in the aboveground parts of the plant can be explained by the fact that Cu is essential metals necessary for plant growth. Like the roots, soil contamination with heavy metals resulted in an increase in metal concentrations in the shoots compared to the reference unit with Cr as exception. The concentrations of the analyzed metals fall within the range of concentrations in agricultural plants from uncontaminated soil, according to Nagajyoti et al., 2010, except for Ni concentration in all model system units, Cd where the soil was treated with Cd, and Pb in the model system unit where the soil was treated with Pb.

Table 3. Heavy metals in shoots (mean ± SD, mg kg^{-1} of dry matter)

Sample	Cd	Cr	Cu	Ni	Pb
Ref-Sh	0.22±0.02	0.61±0.05	6.9±0.2	3.08±0.02	2.2±0.1
Cd-Sh	32.04±0.06	1.05±0.03	6.91±0.03	3.30±0.04	2.3±0.2
Cr-Sh	0.108±0.009	0.61±0.03	7.6±0.2	2.92±0.04	2.3±0.2
Cu-Sh	0.167±0.009	0.300±0.009	11.1±0.3	2.96±0.02	2.86±0.06
Ni-Sh	0.17±0.02	0.39±0.04	6.5±0.2	4.70±0.06	1.7±0.1
Pb-Sh	0.20±0.02	1.06±0.04	8.3±0.2	6.00±0.08	15.5±0.3

The translocation factors (TF-ratio between the concentration of metals in the aboveground part of the plant and in the root) are less than 1 for all elements in all model system units which indicates that the metal content is higher in the root than in the shoot, except for Cu (1.73) where the soil was treated with Cd. Similar results are obtained for alfalfa (*Medicago sativa* L.) (Mitov et al., 2023). The enrichment factor (EF-ratio of metal concentration in roots of plants growing on polluted and unpolluted soils) values are in the range 0.25-31.15. The highest value was observed for Cd in the model system units where the soil was treated with Cd, indicating that white clover can absorb significant amounts of Cd when it is present in higher quantities in the soil.

Conclusion

The concentrations of heavy metals are higher in the roots of white clover compared to the aboveground parts of the plant in majority of samples. Soil contamination with heavy metals leads to an increase in metal concentrations in white clover. Enrichment factors (EF) for the roots, especially in the case of Cd, in the model system unit where the soil was treated with Cd, exhibited high values, indicating that white clover can absorb significant amounts of Cd when it is present in higher quantities in the soil. Treatment of the soil with a mixture of heavy metals at the concentrations applied in the study prevented the growth of white clover.

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THE INFLUENCE OF HEAVY METAL(OID)S SOIL CONTAMINATION ON THE CONTENT OF PIGMENTS IN PEPPER AND MAIZE LEAVES

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Abstract: In this paper, the influence of elevated concentrations of some metal(oid)s (As, Cd, Co, Cr, Cu, Mn, Ni, Pb, and Zn) in the soil on the content of chlorophyll a, chlorophyll b, and carotenoids in leaves of national hybrids of pepper (Bela Duga) and maize (ZP606) was investigated. The results show the most pronounced negative effect of Mn and Pb on pepper, and Mn and Ni in the case of maize. The obtained results can serve in the discussion and better understanding of the potential of pepper and maize to deal with elevated concentrations of these metal(oid)s in the soil and their negative effects on plant growth and development.

Keywords: pepper, maize, metal(oid)s, pigments, UV/VIS

Introduction

Pigments, such as chlorophyll, carotenoids, and flavonoids, play a crucial role in the processes of photosynthesis and the overall health of plants. Additionally, their content in the plants can serve as a potential bioindicator of pollution. Nechitailo et al. (2018) analyzed the influence of Fe, Zn, and Cu on pepper. These metals significantly increase the length and activity of the roots, while Fe and Cu influence the increase of chlorophyll content in pepper leaves. Altaf et al. (2022) examined the physiological and biochemical responses of pepper seedlings to Ni toxicity. Their findings indicate that Ni toxicity induces chlorosis in young leaves, reduces growth and biomass production. Additionally, an excess of Ni enhances the activity of antioxidant enzymes and the accumulation of reactive oxygen species (ROS) in pepper leaves. Cui and Zhao (2011) investigated the influence of Zn and S on the growth of maize. Their research suggests that S mitigates the toxicity of high Zn concentrations and increases the concentration of chlorophyll, while Zn increases peroxide

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species in maize leaves. The moderate addition of S reduces oxidative stress caused by an excess of Zn. Alonso-Blázquez et al. (2015) studied the impact of increased Zn content in the soil on the growth and development of wheat and maize. Zinc inhibited the germination of wheat and the growth of maize. According to these authors, the increase in Zn concentration in the soil contributed to the increase in chlorophyll levels in wheat, while the opposite effect was observed in maize. Additionally, Zn induced an increase in the activity of ascorbate peroxidase in maize, while antioxidant activities were inhibited after 35 days. Glutathione levels increased in wheat and maize after 35 days, suggesting that Zn can induce oxidative stress by disrupting the plant's antioxidant defense system. Xu et al. (2014) investigated the ability of maize seedlings to accumulate Cd and their tolerance to it. Their results indicate that lower concentrations of Cd in the soil decrease glutathione content in the leaves, while higher concentrations increase the activity of antioxidant enzymes. Culicov et al. (2022) explored the impact of copper salts on wheat. The results of this study show that copper salts significantly affect the content of bioactive compounds in plant material and the ultrastructure of plants. Significant damage was observed in the leaves of wheat plants treated with copper salts.

The aim of this research is to investigate the influence of As, Cd, Co, Cr, Cu, Mn, Ni, Pb, and Zn on the content of pigments in the leaves of national hybrids of pepper (Bela Duga) and maize (ZP606).

Materials and methods

Setting up the pot experiment: soil preparation

The soil, previously unused for agricultural production, was mixed with peat (Special substrate, Hawita-Antolin) in a 1:1 v/v ratio. Two kilograms of this mixture were measured for each unit of the pot experiment. Subsequently, the measured soil was treated with solutions containing individual metal(oid)s (As, Cd, Co, Cr, Cu, Mn, Ni, Pb, and Zn). The quantity of Mn in the soil was 0.5×MAC (Maximum Allowable Concentration of metals in soil), while for all other metal(oid)s the concentration in the soil was 1.5×MAC (Official Gazette of the Republic of Serbia, 1994). The treated soil was left in plastic bags for a week with occasional mixing. Prepared soil was transferred into pots, where maize was sown, and pepper seedlings were planted.

Pigments determination

After 120 days for pepper and 76 days for maize the plant material (leaf samples) was collected for the analysis of chlorophyll a, chlorophyll b, and carotenoids. Leaf samples were first washed with tap water and then with distilled water. The washed leaf samples were carefully crushed and homogenized. Approximately 0.5 g of each pepper and maize leaf sample was measured into 100 ml Erlenmeyer flasks, 20 ml of acetone was added, and the mixture was shaken on a shaker for 30 minutes at a rotation speed of 480 rpm, all in complete darkness. Supernatants were carefully decanted through qualitative filter paper, and the entire process was repeated with an additional 20 ml of acetone for 10 minutes. Filtrates were collected in 50 ml beakers and kept in the dark until the spectrophotometric determination of pigments. Pigments contents were determined spectrophotometrically using appropriate equations (Culicov et al., 2022; León-Chan et al., 2017).

Results and discussion

Figure 1 illustrates the concentrations of pigments (chlorophyll a, chlorophyll b, and carotenoids) in the leaves of pepper and maize, cultivated in experimental pot units, where the soil was treated with selected metal(oid)s. Symbols of metal(oid)s on the x-axis denote samples of leaves from agricultural crops treated with these metal(oid)s, while the label “Ref“ refers to a leaf sample from the control unit of the experiment.

It is observed that the concentrations of both chlorophyll a and chlorophyll b are higher in pepper leaf samples compared to maize leaf samples in all experimental pot units. The situation is the same for choretenoids in most experimental pot units (As, Cd, Co, Cr, Cu, Ni, and Ref).

The contents of the examined pigments in pepper leaf samples follow the next sequence: Chl,a>Chl,b>carotenoids. The highest content of all analyzed pigments was determined in samples from experimental units treated with Cr and the smallest in the experimental unit treated with Mn and Pb. Also, the concentrations of pigments in pepper leaf samples harvested from units treated with Cr, As, Cu, Co, Ni, and Zn were higher than in the pepper leaf sample from the control unit i.e. treatment with Pb and Mn reduces the content of these pigments.

The contents of the examined pigments in maize leaf samples follow the next sequence: Chl,a>carotenoids>Chl,b. The highest pigments contents were determined in the experimental unit treated with Zn and the smallest in the experimental unit treated with Mn and Ni. A higher content of chlorophylls and carotenoids, compared to the control unit, was observed in samples from units treated with Zn, Pb, Cu, Cr, and As i.e. treatment with Cd, Co, Mn, and Ni reduces the content of these pigments.

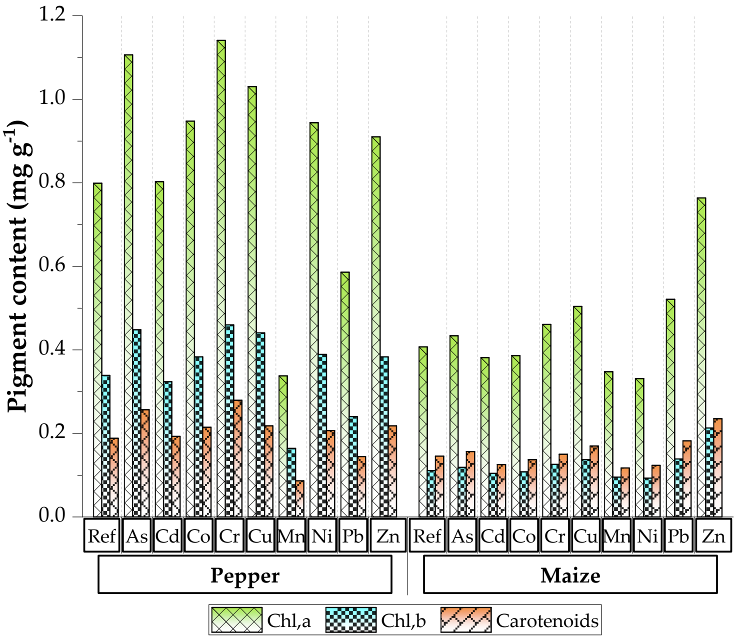

Figure 1. The average content of chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids in the leaves of pepper and maize.

As mentioned, the chlorophyll content in the pepper leaf grown in the copper-treated experimental unit is higher than in the leaf from the control unit, which is in accordance with the results of Nechitailo et al. (2018). Despite Ni showing an extremely toxic effect on pepper seedlings according to the study by Altaf et al. (2022), in this research, it did not induce chlorosis in young leaves even the chlorophyll content in the pepper leaf sample from the pot treated with Ni is significantly higher than in the pepper leaf from the control unit.

Although Cui and Zhao (2011) and Alonso-Blázquez et al. (2014) reported zinc toxic effect on maize, in this study, the content of pigment substances is highest in the maize leaf grown in the zinc-treated pot. Also, in line with the results of Alonso-Blázquez et al. (2014), an increase in Zn concentration leads to an increase in chlorophyll content in wheat leaves, corresponding to the results of this research for both maize and pepper. Xu et al. (2014) reported the toxic effect (leaf spots, biomass reduction) of Cd on maize, which is confirmed in this study, where a lower content of pigment substances was observed in the maize leaf grown in the pot treated with Cd compared to the leaf grown in the reference pot. Researchers from Pakistan observed toxic effects of Mn and Cr on maize expressed through reduced content of chlorophyll a, chlorophyll b, and carotenoids (Shafique et al., 2020).

Conclusion

The results of this research, as well as the results of other authors, show different behavior of different plant species towards selected metal(oid)s in terms of their influence on the content of investigated plant pigments.

The obtained results show that leaves of pepper have a higher content of pigment substances compared to maize. Also, the pepper did not show specific resistance to Mn and Pb, unlike the other tested metal(oid)s. In the case of maize, a decrease in the concentration of pigments was observed in the case of Cd, Co, Mn, and Ni.

The soil treatment with Cr created the most favorable conditions for pepper cultivation, while Zn soil treatment had the most favorable impact on maize cultivation among all the examined metal(oid)s. Also, Mn and Pb show the greatest negative effect in the case of pepper and Ni and Mn in the case of maize.

Future research should be focused on the study of the influence of a higher content of some examined metal(oid)s in the soil, as well as on the correlations between the mass, height, and appearance of the leaves on the one hand and the content of pigments on the other hand.

Acknowledgement

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PHYTOCHEMICAL CONTENT AND ANTIOXIDANT PROPERTIES OF OSMOTIC DEHYDRATED BLUEBERRY, CHERRY AND POMEGRANATE FRUIT

Ognjen Pavlović[1], Tamara Milenković[1], Nevena Barać[1], Miroljub Barać[1], Ivana Sredović Ignjatović[1]

Abstract: : This paper seeks to research the changes in content of bioactive molecules during the process of osmotic dehydration of blueberry, sour cherry, and pomegranate. The study focuses on the total phenolic content, total flavonoids content, total anthocyanins, and antioxidative activity. The analysis of frozen fresh, partially process and dried samples indicated that application of osmotic dehydratation positively influence on preservation of phenolic compounds and antioxidant ability of tested fruits. The findings of this research could have implications for the food industry, particularly in the production of food with added value.

Keywords: osmotic dehydratation, blueberry, sour cherry, pomegranate, phenolics, antioxidants

Introduction

Fruits and vegetables are a valuable source of many nutritive and bioactive compounds. Because fruits and vegetables are perishable, they are difficult to preserved as fresh products. Drying is one of the oldest method to preserv friuts and vegetables and prolong their shelf life (Kamiloglu et al., 2016). Osmotic dehydration has been increasingly used as a pre-treatment process due to the superior sensory properties it offers compared to conventionally dried products (Ahme et al., 2016). In addition, air-drying is often used as an additional step to improve the shelf-life of the treated fruits and vegetables. The concept of functional food is well-accepted in the industry today, and it implies food with added value in the sight of the increasing availability of bioactive molecules such as antioxidants (Temple, 2022; Nowicka et al., 2015). Phytochemicals, which are secondary metabolites of plant metabolism, have been shown to have a beneficial influence on human health. Vitamins,

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flavonols, anthocyanins, betalains, and carotenoids are some well-known examples of phytochemicals that not only give color to the fruit but also possess antioxidative activity (Yalcin and Capar, 2017).

The aim of this study was to investigate the influence of osmotic dehydratation on preservation of bioactive substances in processed fruits. The results of this study will also contribute to a better understanding of the effects of osmotic dehydration on the antioxidant capacity of dried fruits using DPPH and FRAP methods.

Materials and methods

Samples and chemicals

The samples of deep frozen (d/f), semi candied (partially processed, SC) and air-dried (OD) blueberry (*Vaccinium myrtillus*), sour cherry Oblacinska (*Prunus cerasus Oblacinska*) and pomegranate (*Punica granatum)* were obtained from Desing, Serbia. Samples were stored at -20°C until analysis.

All used chemicals were of analytical grade. Gallic acid, quercetin, Folin-Ciocalteu phenol reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), quercetin and 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) from Sigma-Aldrich (Steinheim, Germany); CH_3OH, C_2H_5OH, HCl, Na_2CO_3, NaOH, CH_3COONa, CH_3COOH, $KHSO_3$, $FeCl_3 \cdot 6H_2O$ from Merck (Darmstadt, Germany); $NaNO_2$, $AlCl_3$, KCl, 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 ethanol solution (50%, v/v) as an extraction agent. 1g of frozen non-processed fruit samples were extracted with 6 mL ethanol/water solution in an ultrasonic bath. A mass of 0.5 g of partially processed and osmotically dried samples, respectively, were extracted with 3 mL ethanol/water solution in an ultrasonic bath (Clifton, Series DU-14, North Somerset, USA) for 5 min. The treated samples were centrifuged (Hettich Zentrifugen Universal 32R, UK) for 10 min at 4000 rpm and the supernatant was collected. This extraction procedure was repeated one more time. All supernatants were combined and adjusted to a final volume of 25 mL. Prepared extracts were stored at -20◦C until analysis.

The hypertonic solution used during the osmosis is also analyzed in order to monitor the diffusion of molecules. It was diluted five times prior to analysis.

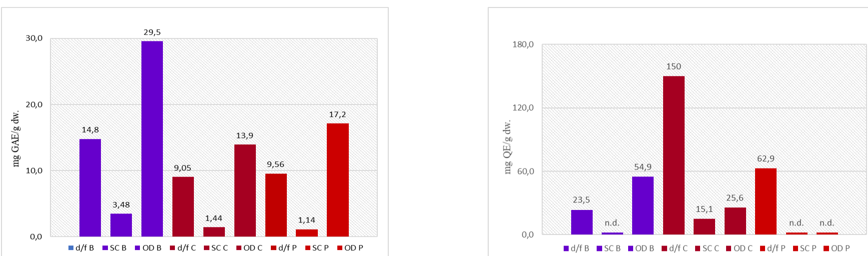
Analytical procedures

The total phenolic content (TPC), total flavonoids (TFC), and total anthocyanins content (TAC) were determined according Milić et al. (2021). TPCs of all samples were expressed as miligrams gallic acid equivalents per gram (g GAE/g) of the dry weight. Total flavonoids were expressed on a dry weight basis as miligrams of quercetin equivalents (QE). Total anthocyanins are shown as mg cyanidin-3-glucoside (C3G) equivalent per gram of dry weight.

DPPH assay and FRAP assay were performed according to Thaipong et al. (2006). DPPH radical scavenging ability of samples were expressed as mmol Trolox equivalent (TE) per 100 grams of dry weight sample. The results of the FRAP assay were expressed as mmol TE per 100 grams of dry weight of the sample.

Results and discussion

In the present study, the focus was on investigating the changes in antioxidative activity and the content of bioactive compounds during osmotic fruit processing. The study analyzed the total phenolic and total flavonoid content of different fruit samples, and the results were presented in Graph 1.

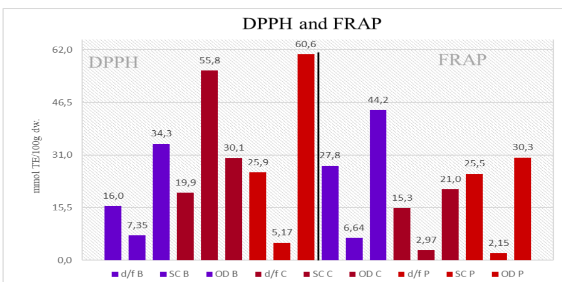


Graph 1. Total a) phenolic and b) flavonoid content
(b – blueberry; c – soar cherry; p – pomegranate)

Results shown on Graph 1 clearly indicated that the osmotically dried blueberry sample had the highest phenolic content, and that the osmotic dehydration process significantly influenced the preservation of these molecules in all analyzed fruit samples. However, the sour cherry samples, which were initially more rich in flavonoids, showed a negative impact of osmosis on the preservation of these biomolecules after undergoing dehydration processing. During osmotic dehydration process a mass transfer occurs, in which moister eluated from fruit to the hypertonic solution, while the solutes flowes into the fruit. Alongside with moister, phenolic compounds, organic acids, micro and macro nutrients also move into the hypertonic solution (Ramya and Jain, 2016). For this reason, TPC decrease in semi candied samples, compared to fresh and dried ones.

Frozen fresh sample of cherry had the highest total flavonoid content, followed with pomegranate and blueberry. In extracts of semi canded samples of blueberry and pomegranate flavonoids was not detected, while dried blueberry was richer in flavonoids than sour cherry. Total flavonoid content in osmotic/air dried cherry was four times higher than in cherries which were vacuum dried (Milić et al., 2021).

The results of antioxidative activity of frozen and dried fruit samples tested using DDPH and FRAP assays are presented on the Graph 2. Blueberries are rich with phenolic acids and flavonoids, vitamins such as C, E, A and micronutrients like Se, Zn, Fe and Mn (Zia and Alibas, 2021b). All of mentioned substances influence the total antioxidant properties of analysed blueberry samples. The pomegranate fruit samples had higher antioxidant activity tested with DPPH than FRAP, while for blueberry samples opposite trend was recorded. The obtained results were in the range and comparable with other authors (Zia and Alibas, 2021a, Benchagra et al., 2021, Can et al., 2022). As in the case of TPC, antioxidant activity decrease in semi candied samples, except for soar cherry sample, in which antioxidative properties toward DPPH were well preserved and concentrated.



Graph 2. Antioxidative properties accesed by DPPH and FRAP methode

The content of total anthocyanins (TA) were determineted using pH differencial method (Table 1). Frozen blueberry sample was richest in anthocyanins and during the drying processes best preserved.

Table 1. Total anthocyanin content (mg / g d.w.)

Sample	TAC	Sample	TAC	Sample	TAC
d/f B	21.19 ± 1.66	d/f C	4.61 ± 0.19	d/f P	1.73 ± 0.12
SC B	3.82 ± 0.21	SC C	0.53 ± 0.03	SC P	0.08 ± 0.002
OD B	23.71 ± 1.33	OD C	2.92 ± 0.43	OD P	0.80 ± 0.16

Analysed frozen blueberry sample had lower anthocyanins content than reported in literature, but after osmotic/air dried dehydratation TA content was higher than those reported for other drying processes (Zia and Alibas, 2021b). Total anthocyanin content after dehydratation decreased in sour cherry and pomegranate for about 1.6 and 2 times, respectively.

Syrups obtained after osmotic dehydratation were also subjected to phytochemical and antioxidant analysis. Results listed in Table 2 indicated that diffusion of bioactive compounds from fruit samples into hypertonic solution occurs during osmotic process. Blueberry syrup was enriched with phenolic compounds, as well as with flavonoids and anthocyanins, comparing to sour cherry and pomegranate syrups. The antioxidant ability of syrups tested with DPPH and FRAP assay had the following trend: blueberry>pomegranate>cherry.

Tabele 2. Syrup's properties

Sample	TPC (mg GAE/L)	TFC (mg QE/L)	TAC (mgC3G/L)	DPPH (mmol Trolox/L)	FRAP
Blueberry syrup	328 ± 21.6	75.8 ± 4.56	18.5 ± 1,44	4.40 ± 0.25	3.99 ± 0.23
Sour cherry syrup	39.8 ± 2.90	39.1 ± 4.40	5.18 ± 0,40	3.58 ± 0.27	2.54 ± 0.19
Pomegranate syrup	39.7 ± 3.37	n.d.	4.09 ± 0.34	4.28 ± 0.36	3.73 ± 0.28

Conclusion

The results showed that frozen blueberry and dried samples contained the highest concentration of bioactive compounds, while sour cherry samples experienced a significant reduction in phytochemical content due to the dehydration process. The antioxidant capacity tested with two methods showed that dehydrated pomegranate sample had the highest activity determined by DPPH assay, while the highest activity tested with FRAP method was in blueberry sample. Analysis of hypertonic solution after osmotic dehydratation indicated that blueberry solution was anreached with phenolic compounds and antioxidants. This study suggests that osmotic dehydration can be an effective method for preserving phytochemicals and antioxidants in fruit samples.

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HEAVY METALS DETERMINATION IN *ACHILLEA LINGULATA* AND *ACHILLEA DISTANS* BY ICP-OES

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Abstract: This paper presents the results of the analysis of heavy metals by ICP-OES method in the inflorescences of the species *Achillea lingulata* and *Achillea distans* subsp. *distans* from the geographical area of the Kopaonik mountain. The presence and content of As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn were determined. A review of the available literature revealed that the heavy metals content in *A. lingulata* and *A. distans* has not yet been investigated. The content of Cd and Pb is below the maximum permissible values in medicinal plants as recommended by the WHO.

Keywords: *Achillea lingulata*, *Achillea distans*, heavy metals, ICP-OES

Introduction

The genus *Achillea* L., which belongs to the Asteraceae family, includes about 100 different flowering plant species, colloquially known as yarrows (Ehrendorfer & Guo, 2006). Inflorescences of *Achillea lingulata* Waldst. & Kit. and *A. distans* Waldst. & Kit. ex Willd. subsp. *distans* (Alpine yarrow) were chosen for the heavy metals content determination.

Materials and methods

All analyses were carried out on an iCAP 6300 Duo inductively coupled plasma optical emission spectrometer (Thermo Scientific Cambridge, UK) with an Echelle optical design and a charge injection device solid-state detector. The iTEVA operating software was used to control all functions of the instrument. Analyzes were performed at the following instrument parameters values: 1150

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W (generator RF power), 50 rpm (peristaltic pump speed), 100 rpm (flushing pump speed), 12 L/min (cooling gas flow), 0.5 L/min (auxiliary gas flow), 0.5 L/min (nebulization gas flow), axial direction plasma observation, three trials for each measurement and wash time between trials 30 s.

Multielement certified standard solution IV (TraceCERT, Sigma-Aldrich, Switzerland: As, Pb (40 mg/L); Fe, Zn (100 mg/L); Cd, Co, Mn (10 mg/L); Cr, Cu, Ni (20 mg/L) was used for preparing working standard solutions for the instrument calibration. Argon (99.999% purity) was purchased from Messer, Serbia. Nitric acid (65%) of analytical grade (Merck, Darmstadt, Germany) was used for complete mineralization of the samples and preparation of blank solution. In order to select working wavelengths, the characteristics of a total of 40 calibration lines for all elements (four wavelengths for each of the elements: As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) were analyzed: relative emission intensity, standard deviation of the slope, standard deviation of the intercept, correlation coefficient (r) and limits of detection (LOD) and quantification (LOQ), interferences to the left and right of the selected wavelength.

The plant material was identified according to standard keys for plant identification as *Achillea distans* Waldst. & Kit. ex Willd. subsp. *distans* and Achillea *lingulata* Waldst. & Kit. ex Willd. (Gajić, 1975). Herbarium specimens are deposited in the Herbarium of the Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš (HMN). The collection of plant material was carried out in June 2021 at the localities of the Mt. Kopaonik: Šiljača, 1642 m a.s.l. (*A, distans*) and Pančićev vrh, 1954 m a.s.l. (*A, lingulata*). The material was dried during the summer in a dark and dry place (average temperature 20°C) and stored under controlled conditions before analysis.

Inflorescences of samples were cut from the stems of dried plants and powdered in mortar. Each sample (1 g) of the plant species *Achillea* was prepared in triplicate by measuring 1 g ± 0.1 mg into Erlenmeyer flasks (100 mL). Then conc. HNO_3 (20 mL) was added to each probe and the solutions were left for 15 min. Further, acid evaporation to dryness was carried out on a sand bath. Then 20 mL of HNO_3 (0.5%) was added to the evaporated probes, which were finally filtered (filter paper Whatman No. 541) into a volumetric flask (50 mL) and filled to the mark with the same nitric acid. Blank probes in triplicate were prepared according to the same procedure.

Results and discussion

The results of the analyses of heavy metals content in the inflorescence of the selected *Achillea* samples are presented in Table 1 and Figure 1.

Table 1. Mean value content of heavy metals in analyzed samples.

Sample	As	Cd	Co	Cr	Pb
	$\bar{x}$ + SD (μg/g DW**)				
AL1	0.912±0.005	0.49±0.05	0.41±0.05	0.45±0.01	2.5±0.1
AL2	0.927±0.005	0.7±0.1	0.45±0.04	0.60±0.05	6.2±0.2
AL3	0.67±0.01	0.80±0.03	0.46±0.03	0.32±0.03	4.73±0.05
AL4	0.64±0.03	0.57±0.03	0.32±0.03	0.27±0.02	0.31±0.05
AL5	0.62±0.05	0.60±0.05	0.35±0.05	0.34±0.04	0.76±0.05
AD1	<LOD	1.19±0.05	0.11±0.02	0.28±0.03	0.6558±0
AD2	<LOD	1.33±0.03	0.17±0.02	0.26±0.01	0.507±0.05
AD3	0.60±0.05	0.97±0.04	<LOD	0.22±0.03	0.4±0.1
AD4	0.7±0.1	1.02±0.03	0.016±0.001	0.28±0.02	0.438±0.005
AD5	0.75±0.03	1.39±0.05	0.081±0.01	0.23 ±0.03	0.51±0.05
Sample	**Cu**	**Fe**	**Mn**	**Ni**	**Zn**
AL1	10.5±0.4	162±15	121±2	4.92±0.05	47±1
AL2	15±2	397±50	149±3	6.4±0.5	52.7±0.2
AL3	16±1	38±3	102±4	3.3±0.2	48±2
AL4	10±1	49.7±0.5	99±5	4.4±0.2	40±1
AL5	12±1	24±3	150±10	4.4±0.5	68±2
AD1	10±1	48.18±0	121±3	10.9±0.5	35±1
AD2	11±1	43±5	115±4	12.8±0.3	34±2
AD3	10.6±0.2	29±3	152±4	10.4±0.2	25.4±0.3
AD4	10.9±0.4	33.47±0.04	87±1	6.4±0.5	34.26±0.004
AD5	8.9±0.5	37±4	72±2	4.2±0.3	22.6±0.2

*AL(1-5)-*Achillea lingulata*; AD (1-5)-*Achillea distan* subsp. *distanss:**DW - dry weight*

The highest content of Pb was determined in samples of the species *A. lingulata* (AL1-AL5), but it is below the maximum allowed values (10 mg/kg) in medicinal plants as recommended by WHO (2007). *A. distans* is characterized by the highest Cd content. The maximum permissible value for Cd in medicinal plants is 0.3 mg/kg (WHO, 2007). The concentrations of Co and Cr less than 1 μg/g were determined in the analyzed samples of yarrows. Iron was determined in the widest range of concentrations from 24 μg/g (AL5) to 397 μg/g (AL2). Compared to *A. distans* samples, the higher Zn content was recorded in the *A. lingulata* species. The lowest Cu concentration was determined in the sample AD5 (8.9 µg/g), while the highest content was found in the sample AL3 (16 µg/g). The content of Ni content in the tested samples ranged from 3.3 µg/g (AL3) to 12.8 µg/g (AD2). Samples of the species *A. distans* (AD1-AD4) are characterized by the highest content of this element.

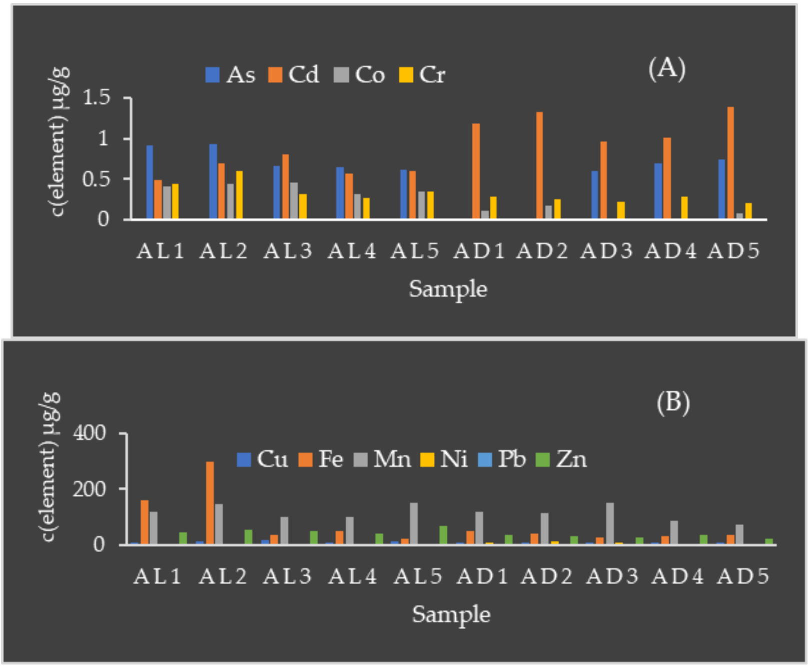

Figure 1. Content (µg/g) of As, Cd, Co, Cr (A) and Cu, Fe, Mn, Ni, Pb and Zn (B) in the analyzed samples of *A. lingulata* and *A. distans* subsp. *distans*.

Conclusion

A review of the available literature revealed that the heavy metals content in *A. lingulata* and *A. distans* has not yet been determined. Table 2 presents a comparison of the results of analyzed elements with the data from some of the published papers that refer to the content of elements in *A. millefolium*.

Hierarchical cluster analysis (Figure 2) showed that based on the heavy metals content all the samples could be grouped into two main clusters. The first cluster includes all samples of *A. distans* and one sample of *A. lingulata* (AL4) due to its similar content of Cr. The second cluster consists of all the other *A. lingulata* samples.

The content of Cd and Pb in the analyzed samples is below the maximum permissible values in medicinal plants as recommended by the WHO.

Table 2. Comparison of the content (µg/g) of the elements with the literature.

Metal	Content	Metal	Content	Species	References
	< 0.15		0.25	*A. millefolium*	Senila, 2014
	/		< LOD	*A. millefolium*	Georgieva et al., 2020
As	/	Cd	0.24	*A. millefolium*	Ražić et al., 2008
	0.62 – 0.927		0.49 – 0.80	*A. lingulata*	This paper
	<LOD – 0.75		0.97- 1.39	*A. distans*	
	/		0.88	*A. millefolium*	Senila, 2014
	/		0.064	*A. millefolium*	Georgieva et al., 2020
Co	/	Cr	0.64	*A. millefolium*	Ražić et al., 2008
	0.35 – 0.46		0.27 – 0.60	*A. lingulata*	This paper
	< LOD – 0.17		0.22 – 0.28	*A. distans*	
	15.7		42.1	*A. millefolium*	Senila, 2014
	10.5		40.29	*A. millefolium*	Georgieva et al., 2020
Cu	9.12	Fe	80	*A. millefolium*	Ražić et al., 2008
	10.5 - 16		24 - 397	*A. lingulata*	This paper
	8.9 - 11		29 – 48.18	*A. distans*	
	83.2		5.14	*A. millefolium*	Senila, 2014
	20.17		0.665	*A. millefolium*	Georgieva et al., 2020
Mn	63.5	Ni	*3.27*	*A. millefolium*	Ražić et al., 2008
	99 -150		3.3 – 6.4	*A. lingulata*	This paper
	72 – 152		4.2 - 12.8	*A. distans*	
	0.68		46.2	*A. millefolium*	Senila, 2014
	<LOD		44.89	*A. millefolium*	Georgieva et al., 2020
Pb	11.59	Zn	22.9	*A. millefolium*	Ražić et al., 2008
	0.31 – 6.2		40 -68	*A. lingulata*	This paper
	0.4 – 0.6558		22.6 - 35	*A. distans*	

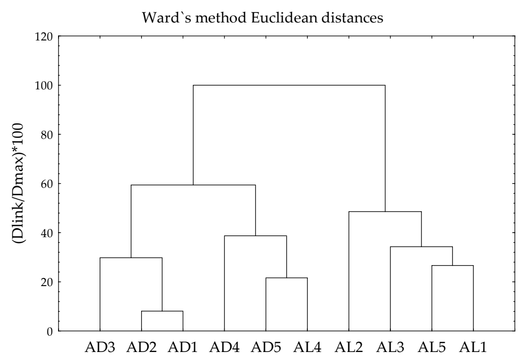

Figure 2. The dendrogram of the cluster analysis of analyzed *Achillea* samples.

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ENHANCING THE EXTRACTION EFFICIENCY OF CAFTARIC ACID FROM GRAPE PULP THROUGHT OPTIMIZATION PROCESS

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Abstract: This study explores the impact of operational extraction parameters, including ethanol concentration, extraction time, and temperature, on the extraction of caftaric acid from grape pulp. Optimal conditions for maximizing caftaric acid content were identified as follows: 20% ethanol concentration, 300 minutes extraction time, and a temperature of 35°C. The investigation employed a full factorial experimental plan (2^3) to assess the influence of these operational factors on caftaric acid content.

Keywords: extraction, experimental plan, operation factors

Introduction

Since the early 20th century, a plethora of studies has investigated the chemical composition of various organs of the grapevine. These studies, spanning from the 20th century to the present day, have consistently revealed the remarkable variability in the chemical makeup among different grape varieties and genotypes (Marais et al., 1991; Kennedy et al., 2001; Zhu et al., 2012). The components under scrutiny encompass a wide array, with polyphenolic compounds taking precedence, followed by organic acids, vitamins, enzymes, carbohydrates, organic nitrogen compounds, terpenoids, volatile compounds, waxes, lipids, polysaccharides, and gums.

The proportion of different components within a grape berry's total mass is subject to variation, primarily influenced by the grape variety and external environmental factors. Among the constituent parts of the ripe fruit, the mesocarp (pulp) constitutes the largest share, ranging from 75% to 90%. Following this, the skin accounts for 9% to 20%, while the seeds represent the smallest fraction, ranging from 2% to 6% (Vujović, 2013). Notably, the

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quantitative distribution of these berry components varies not only across different grape varieties but also within the same variety.

This study seeks to enhance the extraction method through experimental design, aiming to identify the optimal conditions for extracting caftaric acid from the pulp of three grape varieties: black Tamjanika, Smederevka, and Muscat Hamburg. The optimization phase employed a 2^3 experimental design, systematically investigating the impact of key factors—ethanol concentration, extraction time, and extraction temperature—on the caftaric acid content in the extracts obtained under various extraction conditions. Through thorough statistical analysis of the results, conclusions were drawn regarding the influence of the examined factors.

Materials and methods

Materials

For this study, the grape varieties chosen were Hamburg, Smederevka, and Black Tamjanika. In the experimental phase, the pulp extracted from the grape was utilized, having been finely chopped immediately prior to the experiment. The skin and seeds were manually removed and excluded from the study.

Extraction procedure

The extraction experiments were performed using different conditions of solvent concentration, extraction temperature and extraction time. The conditions used in each experiment were settled according to the 2^3 full factorial design (Table 1). After the filtration (filter paper with 6 μm pore size – Whatman, USA), the extracts were stored in the flask.

HPLC analysis

Quantification of individual phenolic compounds was determined using reversed-phase HPLC method. An Agilent-1200 series HPLC with UV-Vis DAD for multi-wavelength detection was used. The column was thermostated at 30 °C. After injecting 5 μL of the diluted plant extract, the separation was performed in an Agilent-Eclipse XDB C-18 4.6 × 150 mm column. Two solvents were used for the gradient elution: A (H2O+2% HCOOH) and B (80 % acetonitrile (ACN) +5 % HCOOH + H2O). The elution programme used was as follows: from 0 to 10 min 0 % B, from 10 to 28 min gradually increases 0–25 % B, from 28 to 30 min 25 % B, from 30 to 35 min gradually increases 25–50 % B, from 35 to 40 min gradually increases 50–80 % B, and finally for the last 5 min gradually decreases 80–0 % B. Flow rate of the mobile phase was 0.8 mL min–1. Caftaric acid present in the extracts were identified by comparing their retention times and spectra with pure component.

Statistical analysis

A full factorial model 2^3 with replication was used to optimize the caftaric acid extraction. The significance of the factors and their combinations were evaluated by the ANOVA using a computer program. The linear first order regression equations were also developed to show the dependence of caftaric acid yield on factors and their interactions.

Results and discussion

In grape pulp, the predominant hydroxycinnamic acid is caftaric acid, in the trans-form. To extract caftaric acid from the pulp of three grape varieties—black Tamjanika, Smederevka, and Muscat Hamburg—maceration, a traditional extraction method, was employed. The study aimed to optimize experimental maceration conditions by investigating the effects of three distinct extraction parameters.

Preliminary tests were conducted to identify the two most influential levels for each tested factor, with significant impact on caftaric acid extraction, mirroring conditions encountered in the wine production process during maceration. These selected levels were subsequently incorporated into the factorial design matrix for the experiments, as showed in Table 1.

Table 1. Eksperimental design and caftaric acid content in grape pulp

	x_1	x_2	x_3	Etanol (%)	Vreme (min)	Tempera. (°C)	BT[1] (μg/g)	S[2] (μg/g)	MH[3] (μg/g)
1	-1	-1	-1	10	120	25	13,74	15,02	6,13
2	+1	-1	-1	20	120	25	15,39	16,91	6,83
3	-1	+1	-1	10	300	25	22,38	22,63	9,53
4	+1	+1	-1	20	300	25	23,67	26,27	10,49
5	-1	-1	+1	10	120	35	20,19	19,62	8,72
6	+1	-1	+1	20	120	35	22,16	21,50	9,53
7	-1	+1	+1	10	300	35	27,96	29,87	13,25
8	+1	+1	+1	20	300	35	30,51	33,52	14,58

[1]BT-Black Tamjanika, [2]S-Smederevka, [3]MH-Muskat Hamburg

The determined caftaric acid content in extract samples 1-8 exhibited a range of 13.75 μg/g to 30.51 μg/g in black Tamjanika pulp, 15.02 μg/g to 33.52 μg/g in Smederevka pulp, and 6.13 μg/g to 14.58 μg/g in Muscat Hamburg pulp. Extract No. 8, characterized by 20% ethanol, extraction time of 300 minutes, and a temperature of 35°C, demonstrated the highest caftaric acid content. In contrast, extract No. 1, with 10% ethanol, extraction time of 120 minutes, and a temperature of 25°C, displayed the lowest caftaric acid content.

The average caftaric acid contents in the pulp of black Tamjanika, Smederevka, and Muscat Hamburg were found to be 22.00 μg/g, 23.17 μg/g, and 9.88 μg/g, respectively.

Gutiérrez-Gamboa and Moreno-Simunovic (2018) determined in Caringan grapes from different regions of Chile that the content of trans-caftaric acid ranges from 24.82 to 57.87 μg/g, while the content of total hydroxycinnamic acids ranges from limits from 51.96 to 130.63 μg/g.

Pajović et al. (2014) determined the content of caftaric acid in the pulp of Vranac, Kratošija and Cabernet Sauvignon grapes. The content of caftaric acid in the pulp of the mentioned grape varieties is similar (16.0 μg/g, 28.4 μg/g, 30.5 μg/g of fresh pulp) to our results. Di Lecce et al. (2014) identified and quantified trans- and cis-caftaric acid in the pulp of Albarino grapes from Spain in the amount of 3.7 and 1.1 μg/g of fresh pulp, which is significantly less compared to our results.

The experimental data of the caftaric acid yield were analyzed with a linear first-order regression model. The statistical significance of all three factors and their possible two- and three-way interaction for the caftaric acid yield were evaluated for their *F*- and *p*-values. The value of $p < 0.05$ indicates the significance of the factors and their interaction. The *F*-values for x_1, x_2 and x_3 factors are in interval 71.858–9289.2, respectively. To simplify the linear regression model, all factors, and interactions, which were assessed to be statistically insignificant with the significance level of 0.05, were omitted; the simplified regression equations are given in Table 2. The most important factor was the extraction time (x_2), which was followed by the extraction temperature (x_3).

Our results showed that the R^2 ranges between 99.88 and 99.90%, indicate that the regression model was suitable for explaining the behavior. Our results showed also that the coefficients of variation were < 2% for all the responses, representing a better precision and reliability of the conducted experiments.

Table 2. Regression equations

BT[1]	$y = 22.00 + 0{,}932x_1 + 4.130x_2 + 3.205x_3 + 0.197x_1x_3 - 0.100x_2x_3 + 0{,}117x_1x_2x_3$
S[2]	$y = 23{,}169 + 1{,}381x_1 + 4{,}906x_2 + 2{,}959x_3 + 0{,}439x_1x_2 + 0.661x_2x_3$
MH[3]	$y = 9{,}882 + 0{,}475x_1 + 2{,}080x_2 + 1{,}637x_3 + 0.097x_1x_2 + 0.060x_1x_3 + 0{,}315x_2x_3$

[1]BT-Black Tamjanika, [2]S-Smederevka, [3]MH-Muskat Hamburg

Conclusion

A full factorial experiment 2^3 was used to determine the optimum parameters that gave a high extraction yield of caftaric acid from grape pulp. The analysis of variance showed that the effects of all variables (ethanol concentration, extraction temperature and extraction time) were extremely significant. The linear regression mathematical models had higher correlation and could be employed to optimize the caftaric acid extraction from grape pulp.

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DETERMINATION OF THE CONTENT OF BIOACTIVE COMPONENTS IN DIFFERENT EXTRACTS OF CELERY LEAVES (*Apium graveolens* L.)

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Abstract: Plants with active compounds, i.e. those that have medicinal properties have been used since ancient times both for medicinal purposes and for preserving food. The aim of the work is to determine the most optimal extraction method for obtaining the highest yield of vitamin C and organic acids from celery leaves, which could have potential application in the food industry. Three extraction methods were combined, the density of the obtained extracts, the content of vitamin C and the content of organic acids were measured. The correlation of the content of bioactive components and the density of the obtained extracts was monitored.

Keywords: extraction, density, content, celery.

Introduction

Celery (*Apium graveolens* L.) is a biennial herb from the Apiaceae family. The plant consists of an upright, ribbed, branched tree up to 1 m tall, with a tail-like thickened root. The leaves are glossy, pinnately compound, with a long stalk, sitting on the stem. The dark green leaves of wild celery reach up to 80 cm in length. The flower stem bears complex shield inflorescences of inconspicuous green flowers speckled with white. The crown leaves are small, white in color. The fruit is up to 1.5 mm long, with two semicircular mericarps. The whole plant, including the tiny brown seeds, is extremely aromatic, (MacVicar, 2006).

Excellent varieties include: French "Dinan" and Dutch "Amsterdam celery for soup". In addition to the listed varieties, the following are characteristic: Chinese celery (*A. graveolens*) - thin stalks that vary from dark green to white in color. It has a distinctive taste; *A. prostratum* – a glossy-leaved creeper that

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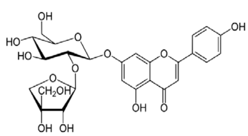
grows on the Australian coast. It is used as a spice in traditional Australian dishes.

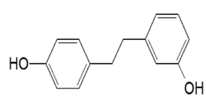
(a) (b) (c)

Figure 1. Celery varieties: (a) celery (*Apium graveolens var.graveolens)*, (b) celeriac (*Apium graveolens var. rapaceum*) and (c) celery leaf (*Apium graveolens var. secalinum*), https://www.google.com/search?sca_1

Celery is characterized by a high content of essential oil. It is mostly found in fruits (2-3%), herbs (0.1%) and rhizomes (0.1%). The main components of the essential oil are: limonene, selinene, r-cymene, β-terpineol, β-pinene and β-caryophyllene (Lajšić and Grujić-Injac, 1998). It is also rich in vitamins (niacin, pantothenic acid, choline, vitamin C and vitamin E) and minerals (potassium, sodium, calcium, phosphorus and magnesium), and it also contains very small amounts of carbohydrates (most of which are dietary fibers), very little fats and proteins (Lakušić, 1990). The plant also contains coumarins, furocoumarin glycosides, furocoumarins, unidentified alkaloids, flavonoids, etc. (MacVicar, 2006). Important constituents of celery are apin, apigenin and lunularin. The components responsible for the smell and taste of celery are butylphthalide and sedanolide (Milić et all.,2012).



(a)



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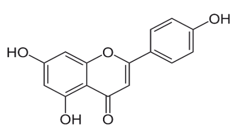

(c)

Figure 2. Chemical formula of (a) apin, (b) apigenin and (c) lunularin

It is consumed as a vegetable in many countries around the world. In Europe, hypocotyl is used as a root vegetable. The leaves have a very strong smell and taste, and they are used as herbs in dried form or fresh leaves are used as an addition to soups. In some states, celery is cultivated for its seeds. Celery seed yields a large yield of essential oils used in the perfume industry. Celery seeds are also used to prepare salt with celery, where dried seeds or celery leaves are ground and mixed with salt, (Džamić, 1984).

Materials and methods

Celery plant (Apium graveolens L.) was used as material in this final work. Extracts for analysis were obtained from the crushed leaf of the plant.
The moisture content is obtained from the difference in mass before and after drying the tested sample, (Damjanović, 2007).

Infusions are aqueous extractive solutions obtained by pouring boiling water over the plant. They are obtained from plants with a more delicate structure or from plants that contain thermolabile, i.e. easily volatile, active principles.

Maceration is a one-time extraction of a crushed plant sample, and it is performed with a suitable solvent at room temperature. In the preparation of the macerate, a cold solvent is used, which reduces the decomposition of active substances, (Aćamović and Cvijović 2009).

Ultrasonic extraction does not require high temperatures, sometimes using smaller amounts of extractants. This type of extraction allows the penetration of the solvent into the intact cells, which increases the yield in a shorter extraction time. Ultrasonic extraction is performed in an ultrasonic water bath, (Greathead, 2003). For the purposes of this work, the density of extracts obtained by maceration, infusion preparation and ultrasonic extraction was read. The hydrometer is immersed in the tested solution, whereby it sinks more or less depending on the density of the liquid. If the density of the tested solution is higher, the hydrometer sinks less and vice versa. The reading is done by aligning the division on the scale with the liquid level in the container, (Piletić and Miletić, 1989). Quantitative determination of total vitamin C is based on the reversible ability of the oxidation-reduction system ascorbic-dehydroascorbic acid, (Šiler, 2009).

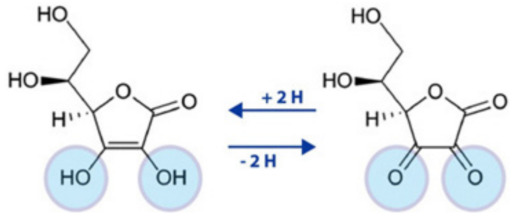

Figure 3. a) L-ascorbic acid and b) L-dehydroascorbic acid

For the quantitative determination of vitamin C, the Tillmans method is used, 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, i.e. Tillmans reagent (TR) is performed in an acidic medium at pH 4-6, (Aćamović and Cvijović 2009).

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 form.

Ascorbic acid content (in mg/10 g extract) = ((V-Vsp) x c x 100)/ Val,

wherein:

V – mean value of TR solution volumes used for the titration of the test sample (ml),

Vsp – mean value of TR solution volumes used for blank titration (ml),

c – titer of TR solution (mg $C_6H_8O_6$/1 mlTP solution) i

Val – volume of an aliquot part of the sample (ml).

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. Free acidity is expressed in g/100 g of fresh sample through citric acid, which is dominant in the sample.

Free acidity (g/100 g) = (V x K x 100)/G, where:

V – volume in cm^3 of used solution 0.1 mol/dm3 NaOH;

K – coefficient for conversion to a specific organic acid (amount of acid in grams corresponding to 1 cm^3 of 0.1 mol/dm^3 NaOH solution);

G – the amount of the tested sample in grams.

Results and discussion

Moisture content and percentage of dry matter in celery leaves. Analysis was performed in three trials. The mean value was calculated from the obtained values.

Table 1. Moisture content and percentage of dry matter

Mass before drying	Mass after drying	Percentage of dry matter
5.00	0.605	12.10
5.00	0.600	12.00
5.00	0.587	11.74

Table 2. Percentage yield and density of extracts

Celery leaf	Infusion	Macerate	Ultrasonic extraction
Percentage yield,%	8.41	12.15	19.5
Density, g/cm^3	0.37	0.42	0.59
Vitamin C, mg/100g	9.45	11.25	16.0
Content of organic acids,g/100g	0.0608		

The content of dry matter obtained after all three measurements was calculated as their mean value and is 11.95%, and the moisture content was obtained by subtracting the content of dry matter per 100 g of sample for each measurement individually and is expressed as their mean value. Moisture content is 88.05%. Based on the obtained results, it can be concluded that the lowest yield was obtained by the infusion preparation method, followed by maceration, and the highest yield was obtained by ultrasonic extraction, (Greathead, 2003). The results are fully justified considering that ultrasonic extraction is the fastest method and requires lower temperatures compared to maceration, while the preparation of infusion is a suboptimal method, as boiling water is used, which breaks down most of the active substances. Based on the results obtained by measuring the density with a hydrometer, it can be seen that the highest density obtained with ultrasonic extracts is 0.59 g/cm^3, which is correlated with the extraction yield. Based on the obtained results, it can be seen that the ultrasonic extraction method is the most optimal, because the yield was the highest, (Yang, 2010). After the analysis, it was determined that the content of total acids in the sample is 0.0608%, i.e. that 100 g of the sample contains 0.0608 g of organic acids.

Conclusion

After the tests were completed, it was determined that the highest content of vitamin S determined by the ultrasonic extraction method was 16 mg/100 g.

The largest amount of this vitamin was isolated using the ultrasonic extraction method because the temperature in the bath was 40°C, which is lower than the temperature of its decomposition (50-60°C). Boiling water was used for the infusion, and the maceration lasted for 5 days, which probably led to some losses, even though the solvent was at room temperature. When taking into account the fact that the ultrasonic extraction method itself lasts only 30 minutes (the shortest of these three methods) and that the operating temperature is 40°C (lower than the decomposition temperature of vitamin C), it is concluded that the vitamin was quickly extracted and preserved until decomposition.

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COW'S MILK IN RELATION TO DIFFERENT PLANT-BASED DRINKS: PHYSICO-CHEMICAL PROPERTIES AND NUTRITIONAL IMPORTANCE

Igor Đurović[1], *Miloš Petrović*[1], *Vesna Matejić*[1]

Abstract: In this study, selected physico-chemical properties of cow's milk were compared with plant-based drinks made from rice, buckwheat, quinoa, almonds, and hazelnuts. Analysis such as conductivity, pH value, viscosity, and density were measured using standardized techniques. Further analysis such as protein, fat, SNF (non-fat solids), lactose, and minerals were determined using a milk analyzer.

The results showed that all plant-based drinks had lower values for protein, SNF, lactose, minerals, pH, and conductivity compared to cow's milk. The other parameters showed a different trend, which is described below.

Keywords: cow's milk, plant-based drinks, physico-chemical properties

Introduction

The term milk refers to a white liquid with a specific taste and odor that is secreted by the mammary gland some time after birth in female mammals and is used to feed the young. Cow's milk has a specific composition that distinguishes it from all other liquids of animal or vegetable origin, which corresponds to its purpose and gives it great biological and certain technological value. Some believe that cow's milk is the perfect food, not only for its nutritional value, but also for its moisturizing properties and its contribution to maintaining the body's overall immunity in infants (Khan et al., 2017).

Cow's milk and dairy products made from it play an important role as a food source with high nutritional content that has a positive impact on human health. The composition of cow's milk can vary considerably depending on many variables such as the breed of cattle, the duration of lactation, the health status of the animal, etc. (Leduc et al., 2021).

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There are numerous studies that provide a complete and exhaustive analysis of the chemical composition of commercial cow's milk at the macro- and micronutrient level. These include numerous data on milk proteins, fats, carbohydrates, various bioactive compounds such as vitamins, minerals, organic acids, biogenic amines, oligosaccharides, nucleotides and immunoglobulins (Fox et al., 2015; Foroutan et al., 2019). Today, milk and dairy products are promoted in public health policies worldwide as an important part of the human diet (Comerford et al., 2021).

Recently, more and more average consumers are consuming plant-based substitutes (Beacomi et al., 2021; Vallath et al., 2022). Plant-based drinks (PBDs) are seen as an alternative to milk and are often sold in stores near dairy products and advertised as having better properties and being healthier than milk and dairy products. There are numerous reasons that can explain these changes in eating behavior. Many authors state that the higher proportion of PBDs on the market is the reason for health consequences such as lactose intolerance (Obermayer-Pietsch et al., 2004), but also the lifestyle in general (vegetarian and vegan) (Antunes et al., 2023; McCarthy et al., 2017).

This work is a continuation of an earlier work in which commercially available plant milks (almond and soy milk) were examined and compared with cow's milk (Milovanović et al., 2023).

Materials and methods

Collection of samples: Commercially available samples such as quinoa, buckwheat, hazelnut, almond, rice, and cow's milk were purchased from a local supermarket.

Preparation of PBDs: 100g of previously ground samples and water were mixed in a ratio of 3:1 in favor of water. The extracts were obtained by ultrasonic extraction at 35 °C for 1.5 hours. After the completion of the extraction, filtration was done, and the physico-chemical properties of the PBDs were further analysed.

Physical analysis: A portable conductivity meter CiberScan CON 510 was used to determine the pH and conductivity of milk and PBDs.

Density was determined by lactodensimeter at 20 °C.

Viscosity was determined using the Ostwald viscometer at 20 °C. The time in seconds was converted to dynamic viscosity 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 the densities of the tested samples and water and η_{H_2O} is the viscosity coefficient of water at 20 °C (Atkins, 1990).

Chemical analysis: The Soxhlet-Henkel method was used to determine the acidity by titrating the sample with 0.25 N NaOH, using 2% phenolphthalein as an indicator until a pale pink color. Acidity is also expressed as % of lactic acid, where each Sokhlet-Henkel degree is equivalent to 0.0225% of lactic acid (Teichert et al., 2020). Proteins, fat, SNF, lactose, and minerals content were determined by milk analyser *LactoStar*. All measurements were performed in triplicate. Results are represented mean values of three independent measurements ± standard deviation (SD).

Results and discussion

The aim of this study was to compare selected physico-chemical analyses of different milk analogues with purchased cow's milk (Table 1). Almond, hazelnut, quinoa, buckwheat, and rice extracts were prepared as milk analogues. Compared to cow's milk, all PBDs had lower values for protein, SNF, lactose, minerals, pH, and conductivity. The highest protein content in PBDs was found in almond and hazelnut extracts at 2.93 and 2.49%, respectively. The highest fat content was obtained in the hazelnut extract (6.52%). This is to be expected as hazelnut is a good source of fatty acids, especially monounsaturated fatty acids, and fat-soluble bioactive compounds (tocopherol, phytosterols) (Wani et al., 2020). As expected, the lactose content is highest in cow's milk (4.61%).

In addition, the results showed a higher conductivity of cow's milk than PBDs. The major contribution to conductivity is made by lactic salts, some of which are dissolved as ions, while others are in equilibrium with various species associated with casein micelles or other proteins, depending on temperature and pH, and their contribution to conductivity is difficult to determine (Henningsson et al., 2014).

Lower density values in PBDs compared to cow's milk were expected due to higher proportion of water in plant-based extracts.

The highest percentage of minerals was found in cow's milk, but when comparing milk analogues, a higher percentage was found in almond and hazelnut extracts than in buckwheat, quinoa, and rice extracts.

Table 1. Selected chemical and physical properties of cow’s milk and plant extracts (rice, quinoa, buckwheat, almond and hazelnut)

Parameters	Raw Cow’s milk	Rice extract	Quinoa extract	Buckwheat extract	Almond extract	Hazelnut extract
Proteins (%)	3.14±0.02	0.72±0.02	1.11±0.01	1.04±0.02	2.93±0.02	2.49±0.02
Fat (%)	3.59±0.02	0.43±0.02	0.12±0.01	0.09±0.02	2.03±0.02	6.52±0.01
SNF (%)	8.57±0.02	1.98±0.02	2.92±0.02	2.82±0.02	7.82±0.01	6.51±0.01
Lactose (%)	4.61±0.02	1.06±0.02	1.63±0.02	1.53±0.02	4.29±0.02	3.50±0.02
Minerals (%)	0.79±0.02	0.22±0.02	0.23±0.02	0.27±0.02	0.45±0.01	0.47±0.02
Acidity (°SH)	7.51±0.02	4.25±0.02	7.74±0.01	4.26±0.01	7.23±0.02	7.50±0.02
Lactic acid (%)	0.17±0.01	0.10±0.01	0.17±0.01	0.10±0.01	0.16±0.01	0.17±0.01
pH	6.15±0.04	5.95±0.03	5.80±0.04	6.03±0.04	5.61±0.03	5.50±0.03
Conductivity (mS)	6.29±0.05	3.07±0.02	5.52±0.03	3.82±0.03	4.74±0.02	1.01±0.02
Density (g/cm^3) at 20 °C	1.030±0.013	1.008±0.010	1.020±0.016	1.020±0.11	1.020±0.012	1.010±0.010
Viscosity (Pa s) at 20°C	1.815±0.022	0.968±0.02	1.121±0.024	3.957±0.032	1.288±0.024	1.606±0.021

Results represent mean values ± standard deviation (SD) of three independent measurements.

The extracts of all PBDs (except buckwheat extract) had a lower viscosity than cow's milk. The buckwheat extract had the highest viscosity. This could be related to the richness of plant fibers, which can cause the formation of large amounts of saponins during ultrasonic extraction. Saponins as a special group of glycosides that easily form foam due to the presence of sugars and triterpenes or steroid compounds and cause high viscosity of buckwheat extract due to pronounced intermolecular interactions.

In terms of acidity (°SH and lactic acid %), the lowest values were found in buckwheat and rice extracts, while cow's milk, almond, hazelnut, and quinoa extracts showed similar values.

Conclusion

The analysis of the tested parameters (proteins, SNF, lactose, minerals, acidity, lactic acid, pH, and viscosity) shows that almond and hazelnut extracts have similar or slightly lower values than cow's milk and have the highest nutritional value of the tested PBDs.

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ANALYSIS OF PHYSICOCHEMICAL PARAMETERS AND TOTAL AMOUNTS OF MICROORGANISMS IN LAKE VLASINA

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Abstract: The comprehensive examination of Like Vlasina microorganism levels and physico-chemical parameters during summer and winter aimed to assess water quality. The temperature ranged from 2 ^{0}C to 24 ^{0}C, pH from 6.20 to 8.40. Key indicators, such as ammonia 0.109-0.387 mg dm^{-3}, nitrates 0.111-0.320 mg dm^{-3}, and nitrites 0.002-0.025 mg dm^{-3}, displayed distinct patterns. The count of mesophilic aerobic hemoorganotrophic microorganisms was lowest in winter 100 and peaked in summer 63500 no x 10^{-3} dm^{-3}
Nitrogen gradients increased with depth. Emphasizing the need for continuous monitoring to prioritize lake protection and address pollution, ensuring its sustained revitalization.

Keywords: microorganisms, temperature, pH, nitrogen gradients

Introduction

Vlasina Lake is located in southeastern Serbia on the territory of the municipality of Surdulica, 30 km from the South Morava valley to the west and from the Serbian-Bulgarian border to the south. It covers the greater part of the Vlasina Plateau. Located at 1,213 m above sea level and covering an area of 16 km^2. Vlasina Lake is the largest and highest artificial lake in Serbia.

Two cubic meters of first-quality water flow into the lake every minute. The water still contains dissolved organic matter, nitrogen compounds, phosphorus compounds, oxygen, etc. in different concentrations, depending on the season and temperature that dictates the movement of the cycle in the lake. It was formed in the place where in the past there was one of the largest mountain valleys of the Balkan Peninsula, better known as the Vlasina mud, i.e. where the Vlasina river stood out.

The lake, whose water color varies from gray-blue near the shore to dark blue in the middle of the lake, with green coastal areas, an indented coast, two

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islands and several elongated peninsulas with rocks, gives a special color to the Vlasina landscape. The Vlasina Lake and its surroundings have a sub-mountainous climate, with short dry, and fresh summers and cold winters. Water quality, great floristic, and faunal wealth are the basic values of this natural asset. In this work, the number of microorganisms present was analyzed, as well as the content of certain physical and chemical parameters to determine the water quality of this lake.

Materials and methods

The samples were always taken at the same places determined by landmarks on land, at different depths, with a prochromic bottle of volume 3 dm^{-3} with a mechanism for opening at the desired depth. They were immediately transported to the laboratory, filtered through a membrane filter with a pore size of 0.45 μm and then analyzed. Water sampling was done in winter (January 2022) and summer (August 2022).

The analyzed water quality parameters are: temperature, pH, oxygen saturation, concentration of nitrates, nitrites and ammonia. The temperature was measured in situ with a Dalmacija DT digital thermometer with an accuracy of 0.050 ^{0}C, the pH value was determined with a Wallace and Tiernan Col 2000 digital pH meter. The oxygen saturation was determined with the titrimetric method according to Winkler.

Concentrations of nitrate, total phosphorus and total organic matter were determined by spectrophotometric methods on a Cintra 404 UV-VIS spectrophotometer.

Analyzes of microorganisms were determined in the same period as the physical and chemical parameters. Microorganisms were determined using the Pour Plate technique on R.2 Agar. Incubation continues 168 hours at 20 °C. The seeded microorganisms were then counted under an electron microscope and expressed as the number of microorganisms per dm^3 of water.

Results and discussion

Table 1 shows the results of the analysis of the vertical distribution of the physical and chemical parameters of the Vlasin Lake water from 6 different sampling locations in January 2022 and August 2022. With increasing depth, temperature, pH and oxygen saturation decrease, while the concentration of nitrogen gradients (ammonia, nitrate and nitrite) is increasing (graph 1).

The highest temperature value measured on the surface of the water in summer was 25 ^{0}C, and the lowest in winter was 30 ^{0}C. The pH values range between 6.20-7.81 for the winter period and between 6.89-8.40 for the summer period, with higher values (more pronounced basic environment) at all stations in the summer period. As a complex expression of the concentration ratio of cations and anions, inorganic and organic ions, aerobic and anaerobic environmental conditions, the pH value of lake water regularly decreases with depth and this characterizes any moment of the test. That vertical gradient is weakest in winter , and reaches its maximum in summer, when it fits into the general stagnation. Nitrate concentration ranged between 0.111 - 0.312 mg dm^{-3}. for the winter period and between 0.109 - 0.320 mg dm^{-3} for the summer period. Nitrite concentration ranged between 0.002 - 0.019 mg dm^{-3} for the winter period and between 0.009 - 0.025 mg dm^{-3} for the summer period. Nitrites are one of the nodal points in the ecological circulation of nitrogen as intermediate products of mineralization on the one hand and denitrification on the other. As very unstable, oxidizable compounds, they can indicate intensive mineralization, i.e. rotting, and hypoxia. For this reason, it is difficult to get into the dynamics, but it is certain that their almost constant presence in a relatively high concentration in the lake is based on intensive processes of detritus decomposition and semi-anaerobic. Ammonia concentration ranged between 0.109 - 0.224 mg dm^{-3} for the winter period and between 0.162 - 1.387 mg dm^{-3} for the summer period. Ammonia nitrogen is present in the entire volume of the lake. The content of microorganisms varied a lot in both test periods (it varied more in the summer period). Its number ranged from 100 to 63500 br x 10^{-3} dm^{-3}.

Table 1. Vertical distribution of some water parameters of Vlasina Lake taken from 6 different sampling sites in January 2022

Mesta uzorkovanja vode	Dubina (m)	Temperatura (ºC)	pH	Saturacija kiseonikom	Amonijak (mg dm^{-3})	Nitrati (mg dm^{-3})	Nitriti (mg dm^{-3})	Ukupni mikroorganizmi (brx10^{-3} dm^{-3})
I	0	3	7,81	81,0	0,185	0,135	0,01	1150
	1	3	7,81	73,6	0,187	0,142	0,012	1150
	3	3,5	7,61	72,8	0,192	0,142	0,016	1180
	6	4	7,50	71,8	0,208	0,155	0,016	1260
	12	5	7,19	66,8	0,224	0,194	0,019	1450
II	0	3	7,79	78,7	0,128	0,122	0,007	1120
	1	3	7,70	75,4	0,131	0,136	0,008	1120
	3	3,5	7,57	62,4	0,147	0,141	0,008	1160
	6	4	7,26	67,1	0,177	0,157	0,008	1200
	12	5	7,11	60,7	0,195	0,174	0,008	1350
III	0	3	7,76	81,6	0,136	0,116	0,008	1100
	1	3	7,67	80,4	0,143	0,121	0,008	1100
	3	3,5	7,61	80,4	0,146	0,147	0,008	1150
	6	4	7,42	76,6	0,168	0,154	0,008	1200
	12	5	7,12	62,6	0,189	0,196	0,015	1450
IV	0	3	7,51	81,2	0,124	0,112	0,002	100
	1	3	7,30	76,4	0,126	0,126	0,008	100
	3	3,5	7,21	71,5	0,148	0,141	0,008	110
	6	4	7,11	67,1	0,153	0,157	0,008	130
	12	5	6,89	60,7	0,174	0,184	0,008	290
	20	5	6,82	58,9	0,181	0,261	0,008	1550
V	0	3	7,43	88,4	0,111	0,122	0,002	100
	1	3	7,33	85,0	0,121	0,126	0,010	100
	3	3,5	7,09	78,6	0,143	0,142	0,012	110
	6	4	6,97	65,7	0,156	0,179	0,008	130

	12	5	6,81	61,9	0,166	0,214	0,008	270
	20	5	6,72	52,3	0,179	0,247	0,009	1550
VI	0	3	7,39	71,8	0,109	0,111	0,01	130
	1	3	7,19	70,4	0,112	0,115	0,013	130
	3	3,5	7,03	68,8	0,126	0,149	0,007	130
	6	4	6,97	62,8	0,146	0,171	0,008	130
	12	5	6,79	58,4	0,149	0,216	0,008	130
	20	5	6,62	51,8	0,178	0,251	0,008	140
	30	5	6,20	49,5	0,201	0,312	0,008	280

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Table 2. Vertical distribution of some water parameters of Vlasina Lake taken from 6 different sampling sites in August 2022

Mesta uzorkovanja vode	Dubina (m)	Temperature (ºC)	pH	Saturacija kiseonikom (%)	Amonijak (mgdm^{-3})	Nitrati (mg dm^{-3})	Nitriti (mg dm^{-3})	Ukupni mikroorganizmi (brx10^{-3} dm^{-3})
I	0	25	8,40	98,0	0,228	0,167	0,013	2010
	1	23	8,21	75,6	0,236	0,198	0,015	3560
	3	23	8,11	62,8	0,298	0,213	0,018	4300
	6	22	8,10	59,8	0,321	0,263	0,018	5230
	12	11	7,79	55,8	0,375	0,294	0,019	33750
II	0	25	8,39	97,7	0,225	0,158	0,009	1980
	1	23	8,37	77,4	0,227	0,196	0,01	2940
	3	23	8,17	57,9	0,248	0,218	0,01	3970
	6	22	8,06	52,3	0,288	0,247	0,01	5090
	12	11	7,53	40,7	0,374	0,287	0,01	31220
III	0	25	8,36	86,9	0,178	0,151	0,011	2020
	1	23	8,27	78,4	0,231	0,156	0,011	3120
	3	23	8,01	71,8	0,257	0,179	0,011	3960
	6	21	7,74	64,2	0,278	0,203	0,011	5120
	12	11	7,42	50,9	0,359	0,258	0,019	32390
IV	0	25	8,11	97,9	0,178	0,121	0,009	3640
	1	23	8,10	87,9	0,234	0,123	0,013	3750
	3	23	8,09	73,8	0,276	0,142	0,013	2870
	6	22	8,00	51,5	0,251	0,185	0,013	2240
	12	11	7,59	43,7	0,359	0,244	0,013	31550
	20	9,5	7,22	40,9	0,396	0,291	0,015	1100
	0	25	8,09	98	0,174	0,111	0,015	3170
	1	23	8,03	88,9	0,211	0,109	0,015	3340
	3	23	8,00	71,9	0,235	0,136	0,019	2990

V	6	20	7,66	65,8	0,299	0,172	0,022	1940
	12	11	7,29	52,5	0,342	0,241	0,022	29670
	20	9,5	7,01	40,7	0,387	0,290	0,025	1090
VI	0	24,5	8,01	96,9	0,162	0,102	0,015	490
	1	23	8,01	89,5	0,191	0,111	0,019	1100
	3	23	7,92	81,7	0,216	0,119	0,021	4300
	6	20	7,81	75,8	0,258	0,168	0,022	6700
	12	11	7,39	69,9	0,288	0,244	0,022	63500
	20	9,5	7,02	61,8	0,318	0,291	0,022	5340

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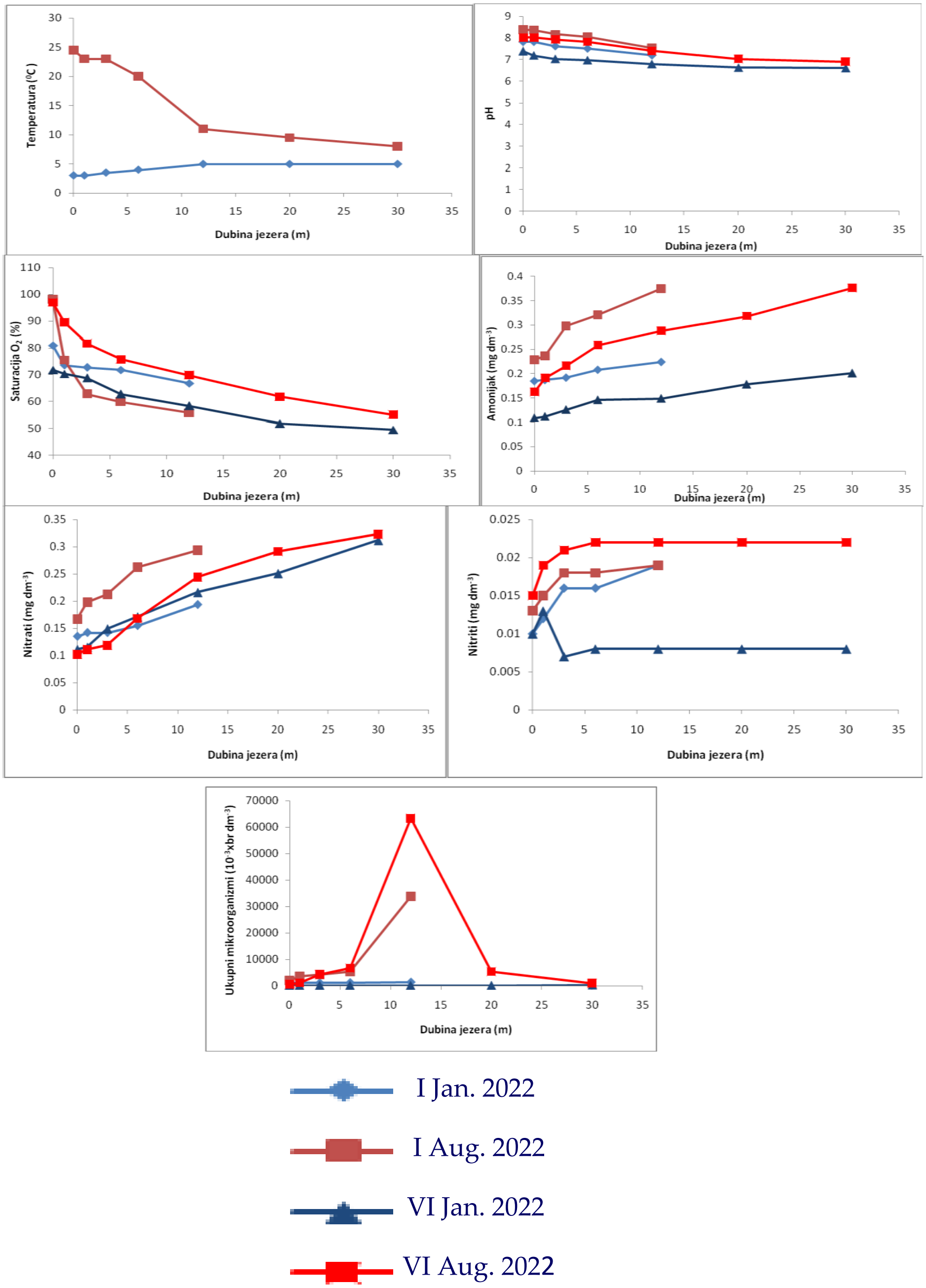

Chart 1: Changes in the physical and chemical parameters of lake water and total microorganisms by depth for two sampling periods, January 2022 and July 2022

Note: Graph 1 shows the values for two stations (I and VI) due to the transparency of the results and their characteristics (these stations differ most in the measured values of the parameters).

Conclusion

The movement of physical and chemical parameters depends on the movement of the water temperature in the lake. It dictates the movement of the cycle in the lake. When the temperatures are uniform, these parameters are also more uniform. (in winter, the values of these parameters change less because the temperatures are more uniform).

Changes in pH following temperature changes. The content of nitrogen gradients (nitrite, nitrate, and ammonia) increases with the increase in the water depth in the lake. Nitrates are consumed in the epilimnion during the summer. Nitrites occur as intermediate products of mineralization on one side and denitrification on the other. These compounds and the present particles mostly settle at the bottom of the lake. The degree of saturation of lake water with oxygen is the result of a complicated relationship between its consumption and renewal. In summer, oxygen is intensively produced in the process of photosynthesis on the surface and is consumed in the hypolimnion. Renewal is done during circulation, but incomplete (deeper layers).

The content of microorganisms is difficult to determine because it changes and is disturbed after each rainfall, its seasonal constancy and vertical distribution cannot be observed. This indicates that the lake ecosystem is very unstable.

The presented data confirm the high quality of the water of the Vlasina Lake, although it is necessary to constantly monitor all physical and chemical parameters as well as the content of the present microorganisms that could threaten the ecosystem of the lake in order to take the most priority measures in the protection of the lakes themselves**.**

Picture 1.: Vlasina Lake

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ANTIMICROBIAL ACTIVITY OF HYBRIDS OF COUMARIN'S DERIVATIVES WITH NEUROTRANSMITTERS

Ivana Radojević[1], Edina Avdović[2], Dušan Dimić[3], Marko Antonijević[2], Marijana Vasić[4], Katarina Ćirković[1]

Abstract: In this study, the antimicrobial activity of five hybrids of 3-acetyl-4-hydroxy coumarin with neurotransmitters was determined: dopamine (1), tyramine (2), octopamine (3), norepinephrine (4) and methoxy-tyramine (5). Antimicrobial activity was assessed using the microdilution method incorporating resazurin, with determination of MIC/MMC concentrations. Testing involved twelve microorganisms, five of which were sourced from mine wastewater. The best activities were shown by substances (1) and (4) on *Bacillus pumilus* and *Pantoea agglomerans*, where the MIC was 31.8 μg/mL. *Pantoea agglomerans* showed the highest sensitivity to all tested substances, except (5). Tested substances didn't show activity on standard strains of bacteria and yeast. In general, substances act above the range of positive controls and have selective and limited activity.

Keywords: antimicrobial activity, standard strains, natural isolates, coumarin hybrids

Introduction

Resistance patterns of pathogenic bacteria to commonly used drugs and antibiotics have become commonplace today, creating disruptions in healthcare worldwide. Natural products, especially phytochemicals, such as curcumin and several others, are often used as antibacterial, antifungal, and anticancer agents in medicinal chemistry. For example, coumarin carboxamide derivative - "novobiocin" and chlorobiocin, aminocoumarin and several others are

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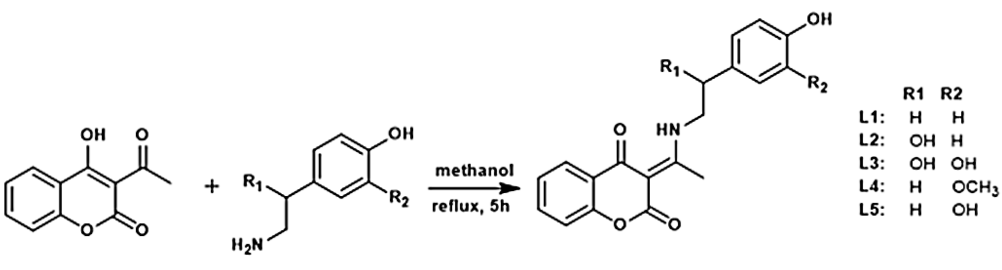
commercial antibiotics. A certain number of antibacterial candidates, coumarin derivatives, were developed by attaching several substituents (for example as functional groups, by incorporating and complexing metal ions in different positions of coumarin, etc.) (Sahoo et al., 2021). Therefore, the aim of this research is to examine the antimicrobial activity of synthesized neurotransmitter-coumarin derivatives.

Materials and methods

All chemicals, 4-hydroxycoumarin, acetic acid, methanol, ethanol, toluene, dimethyl sulfoxide, phosphoroxy chloride, dopamine hydrochloride, tyramine hydrochloride, norepinephrine hydrochloride, octopamine hydrochloride, 3-methoxy-tyramine hydrochloride were obtained from Sigma Aldrich, Germany. The first step is the synthesis of the heterocyclic compound 3-acetyl-4-hydroxycoumarin (Avdović et al. 2021). In the next step, in the reaction of 3-acetyl-4-hydroxycoumarin (AHC) (1 mmol) with different neurotransmitters 1 (2 mmol) (dopamine hydrochloride, tyramine hydrochloride, norepinephrine hydrochloride, octopamine hydrochloride, 3-methoxy-tyramine hydrochloride) were heated in methanol for 5 h (Dimić et al. 2022). The course of the reaction was monitored by thin-layer chromatography. When the reaction was complete, the resulting mixture was cooled to room temperature and the precipitate was collected by filtration. The resulting products 2 were subsequently purified from ethanol (Scheme 1). The neurotransmitters used in this work are shown in Figure 1.



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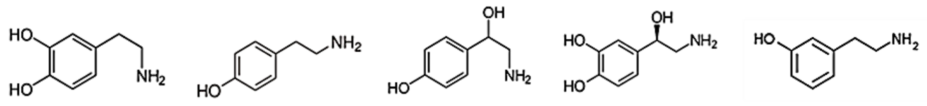
Scheme 1. Synthesis of neurotransmitter-coumarin derivatives



dopamine (1) tyramine (2) octopamine (3) norepinephrine (4) methoxy-tyramine (5)

Figure 1. Neurotransmitters used in this work for the synthesis of coumarin derivatives

In vitro antimicrobial test

Test substances, microorganisms and preparation of the suspension

The antimicrobial activity of the tested hybrid of 3-acetyl-4-hydroxy coumarin with neurotransmitters: dopamine (1), tyramine (2), octopamine (3), norepinephrine (4) and methoxy-tyramine (5) was tested on twelve microorganisms: six standard strains (two gram-positive, three gram-negative bacteria, and one yeast) as well as six isolates from nature (four gram-positive and two gram-negative bacteria) (Table 1). All clinical isolates were a generous gift from the Institute of Public Health Kragujevac. Other microorganisms were provided from the collection of the Microbiology Laboratory of the Faculty of Science, University of Kragujevac. Five isolates originated from mine wastewater. Isolation and identification is described in the work of Branković et al (2022).

Bacterial suspensions were prepared by the direct colony method. The turbidity of the initial suspension was adjusted using a densitometer (DEN-1, BioSan, Latvia). When adjusted to a turbidity of 0.5 McFarland standard (Andrews, 2005), the bacterial suspension contains about 10^8 colony forming units (CFU/mL), and the yeast suspension contains 10^6 CFU/mL. Tenfold dilutions of the initial suspension were additionally prepared in sterile 0.85% saline. Bacterial suspensions were obtained from bacterial cultures incubated for 24 h at 37°C on Mueller–Hinton agar. Yeast suspensions were prepared from two-day-old yeast cultures grown at 30°C on Saburo dextrose agar.

Microdilution method

Antimicrobial activity was tested by determining the minimum inhibitory concentration (MIC) and the minimum microbicidal concentration (MMC) using the microdilution method with resazurin (Sarker et al. 2007). 96-well plates were prepared by adding 100 μL of Mueller–Hinton broth for bacteria and Saburo dextrose broth for fungi. An aliquot of 100 μL of the stock solution of the tested compounds (concentration 2000 μg/μL) was added to the first row of the plate. Two-fold serial dilutions were then performed using a multichannel pipette. The concentration range obtained was from 1000 to 7.8 μg/μL. The method is described in detail in the published paper (Radić et al. 2012).

Doxycycline and fluconazole were used as positive controls. Each test included a growth control and a sterility control. It was noted that 10% DMSO (as a control test solvent) did not inhibit the growth of microorganisms. All assays were performed in triplicate and the MICs were constant (Figure 2).

Minimum microbicidal concentrations were determined by placing 10 μL samples from wells in which no indicator color change was noted, or mycelial growth was not noted, on agar medium. At the end of the incubation period, the lowest concentration without growth (no colony) was defined as the minimum microbicidal concentration.

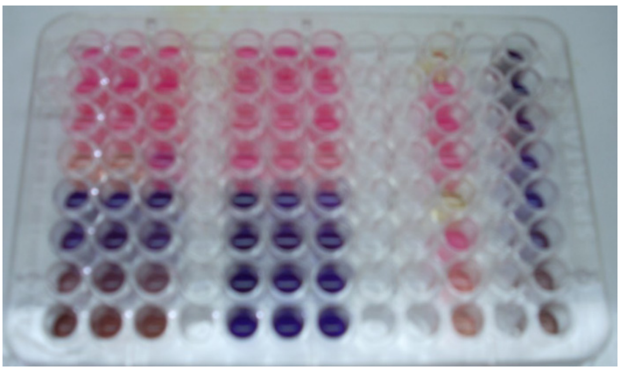


Figure 2. Microdilution method - example of results

Results and discussion

The results of antimicrobial activity testing are shown in Table 1. Coumarin derivatives with selected neurotransmitters have significant antimicrobial activity on isolates from mine waste water. The same tested substances have no activity on the tested standard strains (both gram-positive and gram-negative bacteria, as well as yeast). The minimum inhibitory concentrations and the minimum microbicidal concentrations for standard strains are in the range of 1000/>1000 μg/mL. The best activity was demonstrated by substances 1 (coumarin derivative with dopamine) and 4 (coumarin derivative with norepinephrine) on *Bacillus pumilus* and *Pantoea agglomerans* where the MIC was 31.8 µg/mL. The same substances also work well on isolates of *Bacillus altitudinis* and *Bacillus cereus*. *Pantoea agglomerans* showed the highest sensitivity to all tested substances with the exception of test substance 5 (coumarin derivative with methoxy-tyramine). Generally, the substances act above the range of positive controls and have selective and limited activity.

Table 1. Antimicrobial activity of tested compounds and positive controls

Species	1		2		3		4		5		D/F	
	MIC[1]	**MMC[2]**	**MIC**	**MMC**	**MIC**	**MIC**	**MIC[1]**	**MMC[2]**	**MIC**	**MMC**	**MIC**	**MMC**
Bacillus subtilis ATCC 6633	>1000	>1000	>1000	>1000	>1000	>1000	1000	>1000	>1000	>1000	31.3	62.5
Sarcina lutea	1000	>1000	>1000	>1000	>1000	>1000	1000	>1000	>1000	>1000	<0.5	7.8
Staphylococcus aureus ATCC 25923	1000	1000	>1000	>1000	>1000	>1000	1000	>1000	>1000	>1000	2	15.6
Pseudomonas aeruginosa ATCC 27853	1000	>1000	>1000	>1000	>1000	>1000	1000	>1000	1000	1000	7.812	62.5
Proteus mirabilis ATCC 12453	1000	1000	>1000	>1000	>1000	>1000	1000	1000	1000	1000	125	125
Escherichia coli ATCC 25922	1000	>1000	>1000	>1000	>1000	>1000	1000	>1000	>1000	>1000	1	4
Escherichia coli	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	2	4
Bacillus altitudinis	31.8	31.8	1000	1000	1000	>1000	125	500	62.50	>1000	nt	nt
Bacillus pumilus	31.8	31.8	1000	>1000	>1000	>1000	31.8	500	125	>1000	nt	nt
Pantoea agglomerans	31.8	31.8	62.50	500	31.8	31.8	31.8	250	1000	1000	nt	nt
Bacillus cereus	125	125	1000	>1000	1000	>1000	125	500	250	>1000	<0.5	3.9
Pseudomonas veronii	500	500	1000	>1000	>1000	>1000	1000	1000	>1000	>1000	nt	nt
Candida albicans ATCC 10231	>1000	>1000	1000	>1000	>1000	>1000	>1000	>1000	1000	>1000	0.5	2

[1]MIC values (µg/ml) – means inhibitory activity. [2]MMC values (µg/ml) – means microbicidal activity. nt – not tested; D/F – positive controls.

Acknowledgement

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ANTITUMOR EFFECTS OF *TANACETUM BALSAMITA* ESSENTIAL OILS BY DOWNREGULATION NRF-2 AND MMP-9 IN BREAST CANCER

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Abstract: The aim of this study was to investigate antitumor capacity of different *Tanacetum balsamita* essential oils by measuring apoptosis rate, MMP-9 concetration and Nrf-2 expression level in human MBA-MB-468 and MDA-MB-231 cells. The tested EOs expressed proapoptotic effects, significantly inhibited the MMP-9 concentration and down-regulated the expression level of Nrf-2. The obtained data suggest that the tested EOs exert considerable antitumor activity elevating apoptosis level and inhibiting the motility of tested cancer cells. The reduced levels of Nrf-2 expression suggest decreased defense potential for oxidative disturbances, which could be the major antitumor mechanism detected in the study.

Keywords: *Tanacetum balsamita* essential oils, breast cancer cell lines, Nrf-2, MMP-9, apoptosis rate

Introduction

Cancer represents one of the most persistent groups of diseases with high mortality worldwide. Breast cancer is the second most common cancer of female worldwide. Despite the extensive use of multimodal chemotherapies, their efficiency is limited, emphasizing the need for novel therapeutic approaches with higher cytotoxicity against malignant cells and acceptable demaging outcomes at healthy tissues. Naturally occurring compounds such as terpenoids, phenolics, flavonoids, and alkaloids due to their significant

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therapeutic effects, find increasing medical application in cancer prevention and therapy (Anand et al., 2022). Natural compounds can be used alone or in combination with chemotherapeutics, which allows the application of lower doses of chemotherapeutics in order to overcome drug resistance and toxicity on healthy tissues (Raimondo et al., 2015). Various plant secondary metabolites used in traditional medicine, classified as alkaloids, saponins, terpenes, glycosides and polyphenols, have shown positive anticancer properties *in vitro* and *in vivo*. Accordingly, various products of plant origin, including essential oils isolated from aromatic plants, rise significant interest regarding their anticancer properties (Lin et al., 2019; Sauter, 2020).

Nrf-2 (Nuclear factor erythroid 2- related factor 2) is one of the main regulators of redox homeostasis with predominant antioxidative property and its up-regulation is indicated in breast cancer cell growth and progression. Induction of apoptosis, limits an excessive proliferation of breast cancer cells. Nrf-2 values are used as biomarkers for various specific types of cancer.

Matrix metalloproteinases (MMPs) are proteases with a fundamental role in the degradation of extracellular matrix (ECM) components. MMPs are involved as essential molecules in multiple and diverse physiological processes, such as reproduction, tissue repair, and regulation of inflammatory processes. Its activity is controlled at various levels such as at transcription level, pro-peptide activation level and by the activity of a family of tissue inhibitors of metalloproteinase - endogenous inhibitors of MMPs. Most cancer deaths are the result of tumor dissemination, a process that includes the activity of metalloproteinases. MMPs are used in precision medicine for they role as biomarkers (Mustafa et al., 2022).

The aim of this study was to investigate antitumor capacity of different *Tanacetum balsamita* essential oils by measuring apoptosis rate, MMP-9 concetration and Nrf-2 expression level in human MBA-MB-468 and MDA-MB-231 cell line.

Materials and methods

2.1. Cell culture and treatment

The human breast cancer cell line MDA-MB-468 and MDA-MB-231 were obtained from American Tissue Culture Collection. The cells were grown according to the procedure described in detail in the paper Obradovic et al. (2020). Two essential oils of *Tanacetum balsamita* from different organs (leaf (LEO) and stems (SEO)) were used in experiments. After short-term and long-term treatments (24 h and 72 h), the evaluation of cell proliferation, concentration of MMP-9 and Nrf-2 were performed. The stock solution LEO and SEO was prepared in the concentration of 10 mg mL^{-1}, while the tested concentrations were 1 μg mL^{-1} and 10 μg mL^{-1}. All treatments were performed in triplicate for each assay. After the completed treatments, the concentrations of MMP-9 and Nrf-2 were measured as well as a percentage of apoptotic and necrotic cells.

2.2. Determination of invasive potential of MMP-9 concentration

Matrix metalloproteinases are endopeptidases that play a key role in cell invasion by degrading matrix components such as collagen type IV, native collagen, and others. With the help of a kit for the quantitative determination of the concentration of total MMP-9, it is possible to analyze the invasive potential of cells from cell lysates. This test uses a quantitative sandwich enzyme immunoassay technique. Samples and reagents from MMP-9 (Elabscience, ELISA) must first be prepared as described in the provided instructions. Untreated (control) and treated cells, were trypsinized, centrifuged, and washed three times in PBS. Then cells were sonificated and supernatant was used further in the protocol. Absorbance was measured by using ELISA reader at 450 nm. The concentrations of Human MMP-9 in the samples calculate by comparing the OD of the samples to the standard curve.

2.3. Determination the concentrations of Human NFE2L2 (Nuclear Factor, Erythroid Derived 2, Like 2)

This test uses a quantitative sandwich enzyme immunoassay technique. Samples and reagents from NFE2L2 (Elabscience, ELISA) must first be prepared as described in the provided instructions. Untreated (control) and cell with treatment were trypsinized and then centrifuged and washed three times in PBS. Then cells were sonificated and supernatant was used further in the protocol. Absorbance was measured by using ELISA reader at 450 nm. The concentrations of Human NFE2L2 in the samples calculate by comparing the OD of the samples to the standard curve.

2.4. Determination of type of cell death

Apoptosis and necrosis were analyzed by double staining with annexin V-FITC and 7-AAD. Annexin V binds to the cells with exposed phosphatidylserine, whereas 7-AAD labels the cells with membrane damage. Apoptotic cells were detected using the Annexin V-FITC/7-AAD Kit (Apoptosis Detection Kit, Beckman Coulter, USA). Staining was performed according to the manufacturer's instructions and Shounan protocol (Shounan et al., 1998). After the treatment the cells were collected, washed in PBS and resuspended in ice cold binding buffer. Ten thousand events were analyzed on Flow cytometer Cytomics FC500 (Beckman Coulter, USA). The percent of viable (Annexin V-7-AAD-) cells, early apoptotic (Anexin V+7-AAD-) cells, late apoptotic (Annexin V+7-AAD+) cells, and necrotic cells (Anexin V-7-AAD+) cells were evaluated by Flowing Software (http://www.flowingsoftware.com/).

Results and discussion

The group of matrix metaloproteinases to which MMP-9 belongs, is considered particularly important for the invasiveness of cancer cells during metastasis. In addition to the examination of the migration potential of the cells, in our previus study (Vukic et al., 2022), an analysis of the parameters of the invasion abilities of these cells was carried out. The total concentration of MMP-9 is detected by an enzymatic, ELISA reagent kits method. The results presented on Figure 1. show that LEO and SEO exerted a statistically significant decrease in the concentration of MMP-9 after both treatments compared to the control group of cells. The strongest effect was exerted by LEO at a concentration of 10 μg mL^{-1} on the MDA-MB-231 cell line after long-term treatment (72 h).

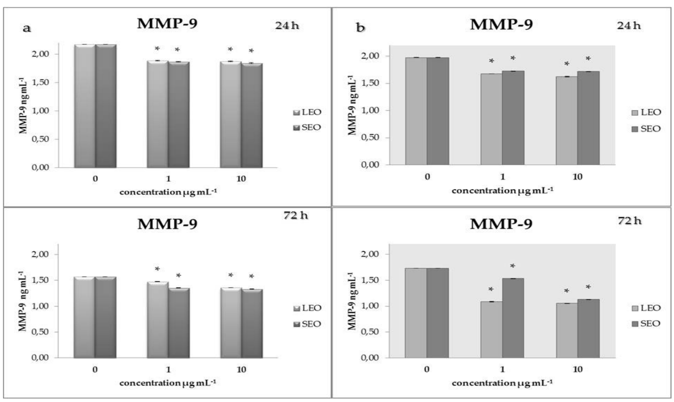

Figure 1. The effects of two concentrations of LEO and SEO on concentration of MMP-9 in a) MDA-MB-468 cells and b) MDA-MB-231 cells after 24 h and 72 h of treatment. Results are presented as the mean of three independent experiments ± standard error; * $p < 0.05$ relative to control.

Cancer biomarkers can play an essential role in fields such as cancer diagnosis and prognosis, monitoring disease progression, predicting disease recurrence, monitoring, and predicting treatment efficacy, as well as cancer screening. MMP-9 is involved as essential molecules in multiple and diverse physiological processes, such as reproduction and regulation of inflammatory processes, but also helps cancer cells to evade primary tumor tissue and migrate across ECM. Since MMP-9 plays an important role in cancer cell progression, it represents a convenient biomarker, already established as such for several cancers (Huang, 2018).

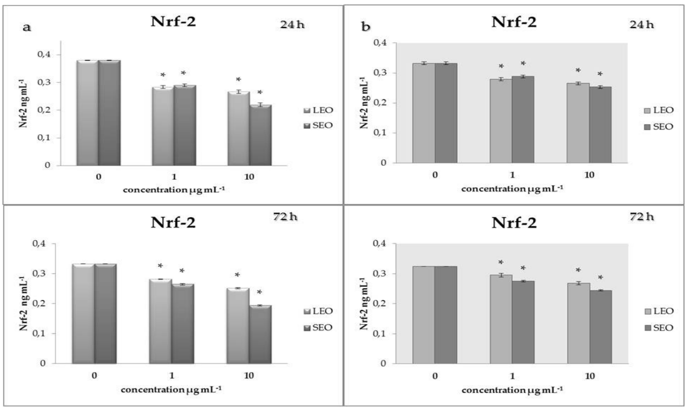

Figure 2. The effects of two concentrations of LEO and SEO on concentration of Nrf-2 in a) MDA-MB-468 cells and b) MDA-MB-231 cells after 24 h and 72 h of treatment. Results are presented as the mean of three independent experiments ± standard error; * $p < 0.05$ relative to control.

In healthy cells, Nrf-2 exerts basic levels of expression, which prevents antioxidative stress and maintaining physiological levels of reactive oxygen species; however, cancer cells overexpress Nrf-2, which is associated with various phenomena, such as the development of drug resistance, angiogenesis, development of cancer stem cells phenotype, and metastasis (Kumar et al., 2022). Redox mechanism-based therapy is known to play an important role in cancer treatment; however, Nrf-2 is responsible for the regulation of antioxidant and cytoprotective properties through the activation of several genes involved in glutathione (GSH) synthesis and chemoresistance. The concentration of total glutathione after 24 h and 72 h of incubation with various concentrations of LEO and SEO showed an increase in the levels of glutathione compared to the control in our previus study (Vukic et al., 2022).

In healthy tissues, Nfr-2 has been extensively considered as one of primary methods for cancer prevention. However, since it exerts cytoprotective action, chemopreventive drugs activate Nrf-2 in cancer cells, and its elevated activity may promote cancer cell survival and proliferation, implying that inhibition of Nrf-2 may be essential pharmacological target in antitumor therapies (Kumar et al., 2022). Nrf-2 targeting cancer progression can provide a new perspective in designing more effective drugs.

The results of our study show that LEO and SEO exerted a statistically significant decrease in the expression level of Nrf-2 after both treatments compared to the control group of cells and presented on Figure 2. The strongest effect was shown by SEO at a concentration of 10 μg mL^{-1} on the MDA-MB-468 cells line after long-term treatment (72 h).

Apoptosis is highly regulated process of cell death and is crucial in maintaining tissue homeostasis, regulating cell division ratio and preventing carcinogenesis. The components of EOs from various plants have been indicated to induce the apoptosis in numerous cancer cell types. Since our previous results suggest decreased viabilty of the tested EOs (Vukic et al., 2022), we have performed the measurments of apoptotic potential as one of the mechanisms of recorded antitumor activity in the study. Apoptosis inducing agents are expected to be sucessful antitumor drugs since apoptosis is protective mechanism against cancer development that acts to remove genetically dameged cells from tissue before they undergo clonal expansion (Goldar et al., 2015).

The type of cell death was determined by flow cytometric analysis of the treated cells stained with Annexin V FITC and 7-AAD. Both cell lines (MDA-MB-468 and MDA-MB-231) were treated for 24 h and 72 h, with concentration 1 μg mL^{-1} and 10 μg mL^{-1}. The effects of LEO and SEO showed statistically significant time- and dose- dependent proapoptotic effect in MDA-MB-468 and MDA-MB-231 cells, as shown on Figure 3. The strongest apoptosis level compared to non tretaed cells was recoreded for LEO essential oil in concentration of 10 μg mL^{-1} in MDA-MB-231 cell line.

The tested essential oils expressed antiproliferative activity, and proapoptotic effects. The investigated oils significantly inhibited the MMP-9 concentration and down-regulated the expression level of Nrf-2.

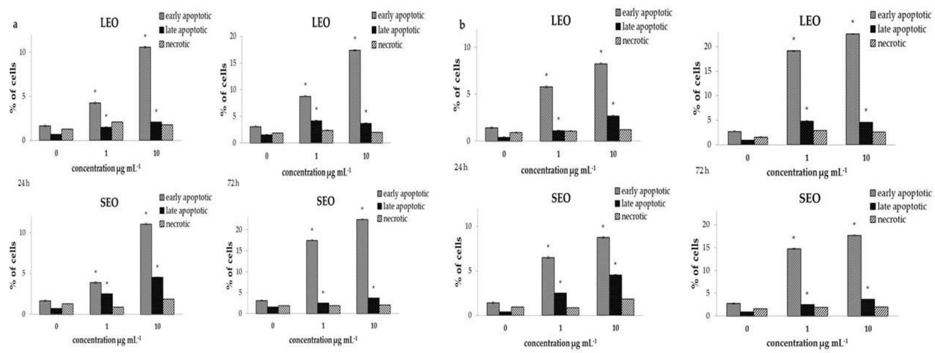

Figure 3. Flow cytometric analysis of Annexin V-FITC/7-AAD stained a) MDA-MB-468 cells and b) MDA-MB-231 cells for 24 h and 72 h with LEO and SEO at two different concentrations. The percentages of early apoptotic (Annexin V+7-AAD-, lower right quadrant), late apoptotic (Annexin V+7-AAD+, upper right quadrant) and necrotic cells (Annexin V−7-AAD+, upper left quadrant) in non-treated and treated cells are indicated on dot plots). Results are presented as the mean of three independent experiments ± standard error; * $p < 0.05$ relative to control.

Conclusion

The obtained data suggest that the tested oils exert considerable antitumor activity by down-regulating Nrf-2 level, elevating apoptosis rate and inhibiting the motility of tested breast cancer cell lines. The reduced levels of Nrf-2 expression suggest decreased defense potential for oxidative disturbances, which could be the major antitumor mechanism detected in the study.

Acknowledgement

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Agreement No. 451-03-66/2024-03/200122 and Agreement No. 451-03-65/2024-03/200122).

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"2nd INTERNATIONAL SYMPOSIUM ON BIOTECHNOLOGY" Proceedings, 2024

INFLUENCE OF EXTRACTION SOLVENTS ON THE PHENOLIC PROFILE OF *ROSA DUMALIS* BECHST. FRUIT SAMPLES

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Abstract: The phenolic content of investigated *Rosa dumalis* fruit samples were studied. To obtain a more detailed phenolic profile, five solvents with different polarities (water, ethanol, 60% ethanol, methanol and acidified 80% methanol) were used for the preparation of the plant extracts. Individual phenolics were analyzed using high performance liquid chromatography (HPLC). The obtained results indicate that the investigated *R. dumalis* samples are rich in both phenolic acids and flavonoids. Acidified 80% methanol extracts had the highest yields followed by the 60% ethanol, water, methanol, and ethanol.

Keywords: *Rosa dumalis*, extraction, phenolic compounds, HPLC

Introduction

The genus *Rosa* L. contains more than 100 species which are distributed natively in Europa, Asia, the Middle East, and North America. Some of the *Rosa* species have a wide range of biological activities such as anti-diabetic, antiproliferative, anti-obesity and antiulcerogenic. This could be a consequence of their phytochemical composition, which include ascorbic acid, phenolics, carotenoids, tocopherols, fatty acids, sugars, proteins, and minerals (Cunja et al., 2016; Demir at al., 2014). Polyphenols are products of the secondary metabolism of plants, and they are important components of both human and animal diets.

Rose hips of *Rosa dumalis* is commonly applied in food manufacturing (Demir at al., 2014; Ercisli, 2007), because of considerable content of phenolics, carotenoids, fatty acids, and vitamins as well as antioxidant potential (Anderson et al., 2011; Bhave et al., 2017; Demir at al., 2014; Nađpal et al. 2018). Growing interest in the utilization of *R. dumalis* in food industry requires detailed characterization of their chemical composition. Selection of an

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appropriate solvent and extraction protocol is the key for successful isolation of biologically active compounds. The polarity of extraction solvents influences the extraction efficiency of phenolic compounds. In general, alcohol, acetone, and water are used for the extraction of bioactive compounds from the plant material (Zhang et al., 2018). On the other hand, the solvents accepted for use in pharmaceutical formulations are water, ethanol, and glycerol (Grodowska and Parczewski, 2010).

The aim of the present work was to investigate influence of extraction solvents on the phenolic profile of *R. dumalis* fruits. To isolate diverse compounds from this plant, five solvents with different polarities were used and all extracts were characterized by their phenolic composition.

Materials and methods

Chemicals

Kaempferol, coumaric acid, gallic acid, (+)-catechin, (-)-epicatechin, rutin, quercetin and procyanidin B2 (HPLC grade), were purchased from Sigma Aldrich (Steineheim, Germany). Cyanidin-3-*O*-glucoside chloride was from ChromaDex (Irvine, CA, USA). Hydrochloric and formic acid were purchased from Merck® (Darmstadt, Germany). Ethanol (96% by vol.), methanol (HPLC grade) and acetonitrile (HPLC grade) were from J.T. Baker (Deventer, The Netherlands). Purified water (18 MΩcm), prepared by a MicroMed purification system (TKA Wasseraufbereitungssysteme GmbH, Niederelbert, Germany), was used to prepare all samples and standards.

Instruments

A model 1200 (Agilent Technologies, Santa Clara, California, USA) was used for HPLC analysis. The analytical column was C18 Zorbax Eclipse XDB-C18, 5µm, 4.6×150 mm (Agilent Technologies, Santa Clara, California, USA).

Samples

Samples of *R. dumalis* were collected during the October 2022 at hill Gorica, Niš, SE Serbia. Due to rugged terrain, four points were chosen on location, with minimum of 500 m distance among the selected rosehip's bushes. Amount of nearly 300-500 g of rosehip was collected on every point. The fruit samples were stored at -20 °C until analysis. Specimens were deposited at the Herbarium of the Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš.

Extract preparation

Approximately 30-40 g of frozen fruit samples were dried by lyophilization. After complete drying, samples were ground to homogenized powder by a grinding mill. Five solvents of different polarity were chosen (water, methanol, acidified 80% methanol, ethanol and 60% ethanol) and the extraction was performed as follows: 1 g of lyophilized sample was weighed accurately and extracted with 20 ml of solvent on laboratory shaker for 60 min and then centrifuged for 10 minutes (4000 rpm). The procedure was repeated for three times. All the extracts were joined and filtered through PTFE microfilter (0.45 μm) and evaporated to dryness under reduced pressure at 40-50 °C. Dry residues were reconstituted in the matching solvent, transferred into 25 ml flask, filled up to the mark and analyzed.

Polyphenols analysis using HPLC

The separation was performed with a Zorbax Eclipse C18 (XDB-C18, 5μm, 4.6×150 mm) column kept at 30 °C, at a flow rate of 0.3 mL min^{-1}, and an injection volume of 20 μL. The binary mobile phase consisted of solvent A (5 % formic acid in water) and solvent B (80 % acetonitrile and 5 % formic acid in water). The 55 min gradient programme, slightly modified, was as follows: 0-28 min 0% B; 28-35 min 25% B; 35-40 50 % B; 40-45 min 80%; followed by 10 min (0% B) of re-equilibration of the column before the next run (Miletic et al., 2022). The individual phenolic compounds were separated within 45 min. Identification was conducted by comparing the retention times and spectral data with those of standards. Quantitative determination of individual phenolic compounds in samples was calculated using calibration lines.

Results and discussion

In Table 1 are shown the results of identification and the quantification of polyphenolic compounds in the analyzed *R. dumalis* samples. In the analyzed fruit samples were identified polyphenolic compounds belonging to the phenolic acid (p-coumaric acid, protocatechuic acid, galic acid), flavanols (rutin, quercetin, kaempferol), flavan-3-ols ((+)-catechin, (-)-epicatechin) and anthocyanins (cyanidin-3-*O*-glucoside, procyanidin B2). The highest amount of galic acid was determined in ethanol extracts (1.22-2.05 μg g^{-1}) followed by the water (0.344-1.24 μg g^{-1}). Galic acid wasn't found in acidified 80% methanol, methanol and 60 % ethanol extracts. Also, p-coumaric acid and protocatechuic acid weren't found in analyzed extracts.

Table 1. Content* (μg g^{-1} dw) of polyphenols in *R. dumalis* extracts

	Solvent	Cy-3-glu	Que	Ru	Kae	Cat	PrB2	Ecat	pCoA	PA	GA
S1	Water	1.81±0.02	nd	3.42±0.04	6.31±0.07	7.30±0.09	22.3±0.5	12.7±0.2	nd**	nd	0.955±0.009
	acid 80%MeOH	1.97±0.02	37±1	3.90±0.04	6.76±0.08	27.6±0.6	51±2	33±1	nd	nd	nd
	MeOH	1.31±0.02	13.2±0.3	2.59±0.03	6.58±0.08	1.09±0.02	5.19±0.07	3.93±0.04	nd	nd	nd
	60% EtOH	1.89±0.02	30±1	3.57±0.04	6.71±0.08	7.83±0.09	44±1	13.4±0.3	nd	nd	nd
	EtOH	1.74±0.02	20.1±0.5	3.27±0.04	6.18±0.07	1.70±0.02	nd	9.7±0.2	nd	nd	1.30±0.02
S2	Water	1.99±0.02	nd	3.55±0.04	6.01±0.07	6.72±0.08	23.9±0.05	10.5±0.2	nd	nd	1.24±0.02
	acid 80%MeOH	1.90±0.02	39±1	3.67±0.04	6.23±0.07	59±2	53±2	62±2	nd	nd	nd
	MeOH	1.69±0.02	10.9±0.2	3.65±0.04	6.73±0.08	2.02±0.02	4.87±0.05	5.87±0.07	nd	nd	nd
	60% EtOH	1.94±0.02	25.8±0.6	3.56±0.04	6.41±0.08	4.98±0.05	39±1	11.9±0.2	nd	nd	nd
	EtOH	1.89±0.02	32±1	3.55±0.04	6.44±0.08	1.21±0.02	nd	9.6±0.2	nd	nd	1.22±0.02
S3	Water	1.88±0.02	nd	3.64±0.04	6.77±0.08	6.41±0.08	27.1±0.6	13.0±0.02	nd	nd	0.529±0.006
	acid 80%MeOH	1.94±0.02	31±1	4.33±0.05	6.92±0.08	30±1	66±2	58±2	nd	nd	nd
	MeOH	1.80±0.02	15.5±0.4	3.49±0.04	6.59±0.08	1.46±0.02	3.80±0.05	4.18±0.05	nd	nd	nd
	60% EtOH	1.91±0.02	23.8±0.5	3.30±0.04	6.63±0.08	6.22±0.08	31±1	17.4±0.4	nd	nd	nd
	EtOH	1.56±0.02	20.1±0.5	3.40±0.04	5.86±0.07	1.96±0.02	nd	10.4±0.2	nd	nd	1.64±0.02
S4	Water	2.07±0.03	nd	3.88±0.04	6.80±0.08	10.1±0.2	27.5±0.6	16.9±0.4	nd	nd	0.344±0.004
	acid 80%MeOH	2.09±0.03	46±1	4.26±0.05	6.99±0.08	41±1	81±3	69±2	nd	nd	nd
	MeOH	1.88±0.02	14.0±0.3	3.62±0.04	6.79±0.08	1.37±0.02	10.3±0.2	3.89±0.04	nd	nd	nd
	60% EtOH	1.96±0.02	28.1±0.6	4.04±0.05	6.32±0.08	5.91±0.07	41±1	13.3±0.2	nd	nd	nd
	EtOH	1.82±0.02	13.6±0.3	3.44±0.04	6.36±0.08	1.31±0.02	nd	10.0±0.2	nd	nd	2.05±0.03

*Values as expressed as the mean value ± SD, n=3. **nd-not detected

Abbreviations: S1, S2, S3, S4-samples of *R. dumalis*; Cy-3-glu-Cyanidin-3-*O*-glucoside; Que-quercetin; Ru-rutin; Kae-kaempferol; Cat-(+)-catechin; PrB2-procyanidin B2; Ecat-(-)epicatechin; pCoA-p-coumaric acid; PA-protocatechuic acid; GA-galic acid.

The highest amount of quercetin, rutin, kaempferol, (+)-catechin, (-)-epicatechin, cyanidin-3-*O*-glucoside and procyanidin B2 were determined in acidified 80% methanol, followed by the 60% ethanol, water, methanol, and ethanol. Rutin (2.59-4.33 μg g^{-1}), kaempferol (5.86-6.99 μg g^{-1}), (+)-catechin (1.31-59 μg g^{-1}), (-)-epicatechin (3.89-69 μg g^{-1}) and cyanidin-3-*O*-glucoside (1.31-2.09 μg g^{-1}) were found in all analyzed extracts. Procyanidin B2 (nd-81 μg g^{-1}) and quercetin (nd-46 μg g^{-1}) weren't found in ethanol and water extracts respectively. Obtained results are in accordance with literature data (Nađpal et al., 2018; Elmastas et al., 2017) or lower (Lakka et al., 2021). Such quantitative differences in *R. dumalis* composition may have been due to different climate conditions, soil composition, cultivation site and technique (Hakkinen, and Torronen, 2000), harvest time (Elmastas et al., 2017) and other factors.

Conclusion

The polyphenolic contents of five extracts with different polarities suggested that yields of acidified 80% methanol extracts were the highest, followed by the 60% ethanol, water, methanol, and ethanol extracts. Among the identified polyphenols, in the highest amount were present catechin and epicatechin in all extracts as well as quercetin and procyanidin B2, except water and ethanol extracts, respectively. Also, the *R. dumalis* fruits have high content of kaempferol and rutin. Due to the polyphenolic compounds, including anthocyanins, flavanols, flavan-3-ols and phenolic acid, *R. dumalis* fruits can be a valuable raw material to use in food and pharmaceutical industry.

Acknowledgement

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EFFECTS OF STANDARDIZED *ARONIA MELANOCARPA* EXTRACT ON CARDIOVASCULAR SYSTEM: FROM BASIC TO APPLIED INVESTIGATION

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Abstract: This study summarizes the effects standardized Aronia melanocarpa extract (SAE) on different pathophysiological processes linked with cardiovascular system from both animal and human investigations. In basic researches we estimated the influence of SAE on rats with metabolic syndrome (MetS) and polycystic ovary syndrome (PCOS). Human studies aimed to assess the impact of SAE in patients with MetS and in professional handball players. SAE had the ability to lower blood pressure and exert benefits on *in vivo* and *ex vivo* heart function. Furthermore, SAE induced a wide range of beneficial effects in human researches. Overall, SAE could be a promising strategy to reduce cardiovascular risk.

Keywords: standardized Aronia melanocarpa extract, cardiovascular system, rats, handball players, oxidative stress

Introduction

Aronia melanocarpa (*A. melanocarpa*) or black chokeberry is a fruit/plant which belongs to the *Rosaceae* family and is native to North America (Daskalova, 2015). However, it has been commonly used in Europe as ingredient for juices, wine, jams, teas and cordial liqueurs (Ochmian, 2012). *A. melanocarpa* represents one of the richest sources of polyphenols among fruits, with anthocyanins and flavonoids identified as major components responsible for its therapeutic potential (Cebova, 2017). Recent researches have focused attention on *A. melanocarpa* due to its numerous health benefits in a broad range of pathological conditions (Jurikova, 2017). It has been reported that fruit and extracts of *A. melanocarpa* exert gastroprotective, hepatoprotective, antiinflammatory and antiproliferative activity (Lupascu, 2016). Furthermore,

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the health-promoting effects of extracts of this plant involve antiatherosclerotic, antiplatelet and hypoglycemic properties (Borowska, 2016). However researches of standardized extract of this plant have not been yet investigated both on animal and human models.

This study summarizes the effects standardized Aronia melanocarpa extract (SAE) on different pathophysiological processes linked with cardiovascular system from both animal and human investigations.

Materials and methods

Ethical aspects

The protocol was approved by the Ethical Committees for animal (119-01-5/14/2017-09) and human investigations (03256/2016).

Study design

The study includes a wide range of research on animal models and on human population.

1. In the first part of the study we examined the effects of different dietary strategies, high-fat (HFd) or standard diet (Sd) alone or in combination with standardized oral supplementation (0.45 mL/kg/day) of Aronia melanocarpa extract (SAE) in rats with metabolic syndrome (MetS). SAE is an official product of pharmaceutical company Pharmanova (Belgrade, Serbia). Rats were divided randomly into six groups: control with Sd, control with Sd and SAE, MetS with HFd, MetS with HFd and SAE, MetS with Sd and MetS with Sd and SAE during 4 weeks. At the end of the 4-week protocol, cardiac function and liver morphology were assessed, while in the blood samples glucose, insulin, iron levels and systemic redox state were determined.

2. In the second part of the study we investigated isolated and synergistic effects of Standardized Aronia melanocarpa extract (SEA) and Metformin (MET) for alleviating reproductive and metabolic PCOS abnormalities. PCOS induction was followed by 28-day treatment with MET, SAE, or MET + SEA. Bodyweight (BW), cyclicity, histological, and ultrasonographical ovarian analyses were performed. Hormonal, glycemic, and lipid profiles were accessed, as well as systemic and ovarian oxidative status.

3. In the third part of the study we examine the effect of 4-week supplementation of Alixir 400 PROTECT® (Standardized Aronia L.

Melanocarpa Extract Extract-SAE) on clinical and biochemical parameters in patients with confirmed metabolic syndrome (MetS). This study was designed as a prospective open-label clinical case-series study with 28 days of follow-up with cases selected and followed during the period from February 1, 2018 to November 2019. The study included 143 male and female patients with MetS who were subjected to SAE.

4. In the fourth part we investigated the effects of one-month consumption of polyphenol-rich standardized Aronia melanocarpa extract (SAE) on redox status in anemic hemodialysis patients. The study included 30 patients (Hb < 110 g/l, hemodialysis or hemodiafiltration > 3 months; > 3 times week). Patients were treated with commercially available SAE in a dose of 30 ml/day, for 30 days. After finishing the treatment blood samples were taken to evaluate the effects of SAE on redox status. Several parameters of anemia and inflammation were also followed.

5. In the last part we investigated the influence of twelve-week consumption of chokeberry extract on redox status, body composition, lipid profile and biochemical parameters in active handball players. The study included 16 handball players, aged 16-24 years (20.26±2.86 years). Every morning before training players received 30mL of liquid chokeberry extract for 12 weeks, during regular competition season. The research consisted of morphofunctional and biochemical testing, which was performed at three points (at the beginning of the study, 6 and 12 weeks after extract consumption).

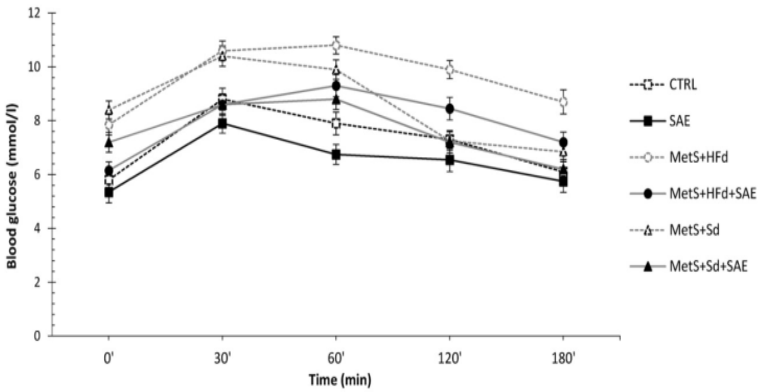
Statistical analysis

Data were collected and analyzed using the Statistical Package for the Social Sciences (SPSS). Descriptive analysis was used to describe results (mean, standard deviation, standard error of mean, frequency in percentage). $\chi 2$ and correlation tests were applied to analyze continuous and categorical variables, respectively. For continuous variables, we used ANOVA for repeated measures (RM-ANOVA). P-value < 0.05 was considered statistically significant.

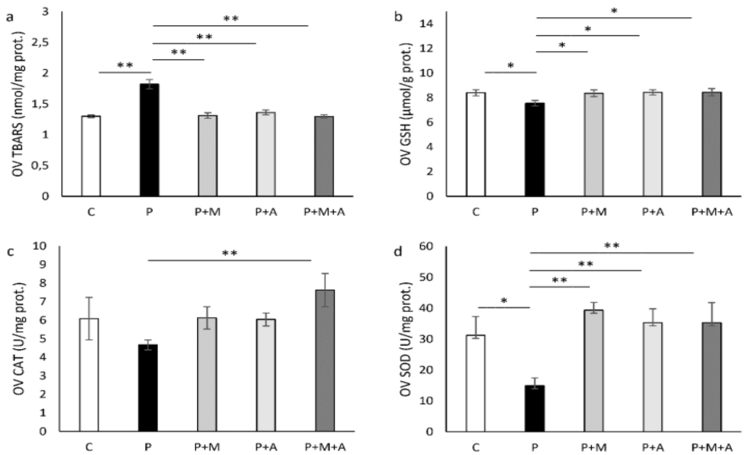
Results and discussion

Results from the first part of the study demonstrated that SAE exert benefits on *in vivo* heart function (Graph 1). Moreover, SAE improved glucose tolerance, attenuated pathological liver alterations and oxidative stress present in MetS. Obtained beneficial effects of SAE were more prominent in combination with changing dietary habits.

In the second part of the study ovarian TBARS levels were increased in the P group ($p < 0.01$) in comparison to the C group. However, treatment with SEA, MET, or their combination decreased these parameter values ($p < 0.01$) compared to the P group (Graph 6a). As shown in Graph 2b, GSH values were decreased in the P group compared to all other investigated groups ($p < 0.05$). CAT activity was increased only in the P + M + A group ($p < 0.01$) compared to the P group (Graph 2c). Ovarian SOD activity was decreased in the P group ($p < 0.05$) in comparison to the C group, and all treated groups showed elevation of SOD compared to the P group ($p < 0.01$), as shown in Graph 2d.



Graph 1. The effects of SAE on glucose levels during OGTT.



Graph 2. Oxidative stress parameters in ovarian tissue

In the third part of the study we found that cholesterol levels significantly decreased in the fMetS-DM group compared to the baseline values in this group, while the LDL levels significantly decreased in the fMetS group. Triglycerides significantly decreased only after 4 weeks of SAE treatment in diabetic groups of patients (fMetS-DM and mMetS-DM) compared to the baseline, while in non-diabetic groups this marker was not significantly altered.

In the fourth part of the study SAE did not improve inflammatory status, except for minor decrease in C-reactive protein. The consumptionof SAE regulates redox status (reduce the productions of pro-oxidative molecules and increase antioxidant defense) and has beneficial effects on anemia parameters.

In the last part of the investigation we noticed that supplementation with chokeberry extract decreased the levels of pro-oxidants (TBARS and nitrites) and increased catalase activity in handball players.

Conclusion

Taken all together, in can be concluded that SAE supplementation was associated with beneficial cardiovascular effects in both animal and human studies through wide range of possible mechanisms such as anti-oxidative potential. Nevertheless all these promising results in triggering cardioprotection should be further examined in future.

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INSIGHT INTO SELECTED QUALITY PARAMETERS OF MEDLAR (*MESPILUS GERMANICA* L.) FRUIT GROWN IN SERBIA

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Abstract: Medlar (*Mespilus germanica* L.) is considered as underutilized fruit, it is nutritionally valuable rich in both primary and secondary metabolites**.** In this work the physicochemical composition and antioxidant properties of medlar fruits originated from different locations in Serbia were examined. The moisture and protein contents of the fruits were in the ranges 73-79% and 0.43-0.47% w/w, respectively. The aveage total phenolic content and antioxidant activity measured by DDPH and FRAP assays, were 2131.62 μg/GAE/g, 1306.6 μmol TE/g and 916.46 μmol Fe^{2+} eq, respectively. Chlorogenic acid (9.61-11.62 μg/g) was the most abundant phenolic acid, whereas contents of catechin (9.69-11.51 μg/g) and epicathenine (7.29-25.31 μg/g) were the highest in most of samples.

Keywords: medlar, underutilized fruit, cholorgenic acid, catechin, quercetin

Introduction

Medlar (*Mespilus germanica* L.) is considered as one of the most underestimated and underutilized fruits among Rosaceae family. For centuries medlar was a common fruit tree in central European gardens (Baird and Thieret, 1989), and until the 17th century it was reported as the most important fruit crop. Nevertheless, interest in the consumption and use of this fruit has steadily diminished and later it was replaced by other, more productive, crops (Popović-Djordjević et al., 2023).

Since relatively recently, medlar fruit is regaining its *'Medieval glory'* and commercial importance as a fruit and food product for human consumption. The traditional uses of medlar fruits were seen both in the gastronomy and

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medicine. From the food production perspective, ripe medlar fruits are mostly consumed as fresh, but they may be processed into different products including juice, concentrate, jam, cheese and honey (Ercisli et al., 2012; Popović-Djordjević et al., 2023).

Medlar fruit has been mostly examined for its nutritional composition (Ercisli et al., 2012; Mikulic-Petkovsek et al., 2023) including the phytochemical composition predominately the flavonoid and phenolic acid content (Mikulic-Petkovsek et al., 2023; Popović-Djordjević et al., 2023).

Studies examining the nutritional quality and phytochemical composition of medlar grown in Serbia are scarce. Therefore, the aim of this study was to explore the chemical composition and antioxidant properties of fruits originated from different locations in Serbia.

Materials and methods

Plant material - *Mespilus germanica* is a deciduous tree which grows up to 6 m. The species has both male and female organs and is self-fertile. It flowers from May to June, whereas the seeds ripen in November when the fruit is harvested. The fruit is round or pear-shaped, and yellow-green to dark brown in color, and it has five large seeds and a cup at the top (Figure 1). When rotted or frozen, the fruits become soft, sweet and juicy (Popović-Djordjević et al., 2023).

Figure 1. Morphological features of *Mespilus gemanica* L. (Photo by N.M.)

Medlar fruits were harvested, in the stage of full maturity (between 9th and 12th November 2022), at 5 locations in the areas near Dolovo (South Banat District;

44° 55' N/20° 52' E; samples 1, 2 and 5), Čačak (Moravica District; 43° 50' N/20° 24' E; sample 3), Smederevo (Podunavlje District; 44° 36' N/20° 59' E; sample 4).

Moisture content (MC) - Moisture was determined gravimetrically, follwoing the previously establosehed protocols (AOAC, 1999).

Protein content (PC) - Protein content was determinate by the Kjeldahl method and calculated by multiplying the total nitrogen by 6.25 (Magomya et al., 2014)

Extraction of bioactive compounds - Bioactive compounds from medlar fruits (2.5 g per sample) were extracted in a mixture of methanol and 10% HCl (10 mL) in a ratio of 85:15%. The procedure was carried out in an ultrasonic bath for 30 minutes. Extracts were filtered using 45 μm syringe filters and stored in a refrigerator (4°C) until analysis.

Total phenolic content (TPC) - TPC of medlar extracts was measured spectrophotometrically (765 nm) using Folin-Ciocalteu reagent and gallic acid as a reference standard. All TPC values are expressed as mg gallic acid equivalents per gram of dry matter (mg GA eq/g dm) (Wong at al., 2006).

Determination of antioxidant activity - Antioxidant activity of medlar extracts was determined using two assays, 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) and ferric reducing antioxidant power (FRAP). The results were expressed as μmol of Trolox Equivalents per gram of dry matter (μmol TE/g dm) and μmol of Ferrous equivalents per gram of dry matter (μmol Fe^{2+} eq/g dm) in $DPPH^{\cdot}$ and FRAP assay, respectively (Wong at al., 2006).

Determination and quantification of individual phenolic compounds - The content of individual henolic compounds (polyphenolic acids and flavonoids) in medlar samples was determined by high-pressure reverse-phase liquid chromatography (HPLC) connected to UV detector (Shimadzu Nexera XR system) (Luthria et al., 2006).

Results and discussion

The results protein content (PC) of the fruits was in the range of 0.43-0.47% (Figure 2A), whereas the moisture content (MC) ranged between 73-79% (Figure 2A), with the highest MC recorded in sample 1. The obtained results for MC were in line with the previous literature (Ercisli et al., 2012). Furthermore, the PC values were similar to the previous results of Al-Amoudia et al. (2013) but somewhat lower than with other studies (Ercisli et al., 2012; Kalyoncu et al., 2013).

The total phenolic content (TPC) in the fruts varied between 1376.94 (sample 3) and 2894.99 μg/GAE/g (Sample 1) (Figure 2B). Similar results were reported in literature (Tessa et al., 2021; Katanić Stanković et al, 2022).

The antioxidant activity, were in the ranges 1057.56-1637.83 μmol TE/g for DPPH$^{\cdot}$, and 603.96-1138.25 μmol Fe^{2+} eq, for FRAP assay. Although sample 1 was richest in TPC, it showed the lowest DPPH$^{\cdot}$ scavenging activity. On the other hand, sample 3 had the lowest TPC and lowest antioxidant activity measured by FRAP assay. Sample 4 expressed the highest antioxidant activity in both assays, Figure 2B.

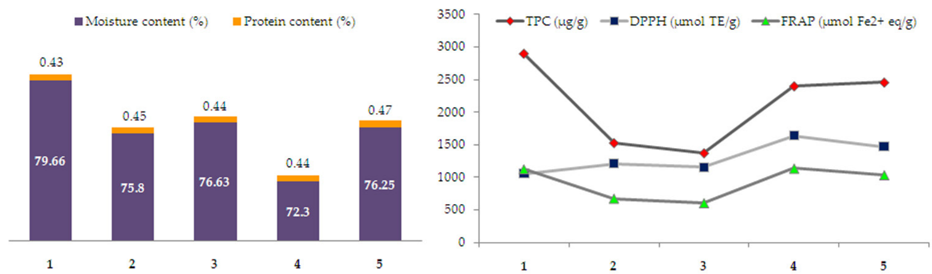

Figure 2. Contents of moisture and proteins (A), total phenolic content (TPC) and antioxidant activity (DPPH$^{\cdot}$ and FRAP) (B) in medlar fruit samples 1-5

Phenolic compounds are a distinguishing class of bioactive compounds distributed in several fruits and commonly associated with a number of beneficial health effects (Popović-Djordjević et al., 2023). The results on phenolic profile revealed that the fruits were rich in several phenolic acids: galic acid (9.05-10.79 μg/g), cholorgenic acid (9.61-11.62 μg/g), caffeic acid (9.12-9.38 μg/g), *p*-coumaric acid (8.30-8.37 μg/g), ferulic acid (8.83-8.65 μg/g), dihydrocaffeic (8.22-8.54 μg/g), phloretic acid (9.00- 14.47 μg/g) and isoferulic acid (8.57- 8.62 μg/g) (Figure 3A).

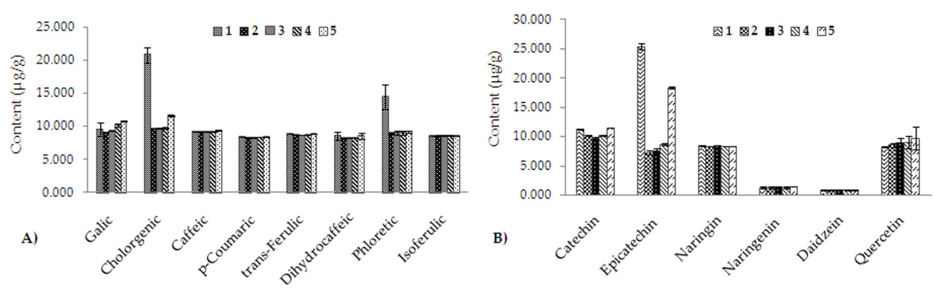

Figure 3. Phenolic acids (A) and flavonoids (B) contents in medlar fruit samples 1-5

Among flavonoids catechin (9.69-11.51 μg/g), epicatechin (7.29-25.31 μg/g), naringin (8.31- 8.45 μg/g), naringenin (1.24-1.49 μg/g), daidzein (0.84-0. 89 μg/g) and quercetin (8.24 -9.71 μg/g), (Figure 3B), were detected in the medlar fruits. The similar phenolic profiles for medlar were reported previously (Katanić Stanković et al., 2022; Mikulic-Petkovsek et al., 2023; Popović-Djordjević et al., 2023). Although the presence of the isoflavones (daidzein) is characteristic of legumes and soybeans, the literature points out the presence of trace amounts of these phenolics in Rosaceae family as well (Sajid et al., 2021). In all studied samples, daidzein was measured in quantities less then 1 μg/g.

Conclusion

The knowledge about nutritional and chemical properties of the medlar fruits grown in Serbia is an essential in order to regain attention to this fruit tree that may lead to restoration of its cultivation and consumption. Obtained results indicate the potential of studied fruits as valuable natural source of bioactive compounds providing the opportunity for its application in the food industry for the development of value-added foods.

Acknowledgement

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CARDIOPROTECTIVE EFFECTS OF LADY'S BEDSTRAW EXTRACT: FOCUS ON OXIDATIVE STRESS

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Abstract: Beside the widespread traditional use of Lady's Bedstraw in the treatment of numerous diseases and conditions, it's effects on heart function and redox status has still not been fully clarified. The aim of our study was to examine the effects of methanol extract of Lady's Bedstraw on oxidative stress parameters during on ischemia-reperfusion (I/R) injury in isolated rat heart**.** Our results demonstrated that treatment with methanol extract of Lady's Bedstraw preserved diminished production of many prooxidants. Promising potential of Lady's Bedstraw in the present study in a model of pharmacological preconditioning may be a starting point for future researches.

Keywords: *Lady's Bedstraw*; ischemia-reperfusion injury; oxidative stress; rat heart

Introduction

Acute myocardial infarction (AMI) continues to be a prominent cause of hospital admissions and mortality worldwide. The current therapeutic focus revolves around promptly restoring blood flow through thrombolytic therapy or primary percutaneous coronary artery angioplasty, emphasizing its paramount importance in mitigating the extent of myocardial infarction (Xia et al. 2016).

However, the reestablishment of blood flow to oxygen-starved myocardium during reperfusion paradoxically intensifies tissue injury induced by hypoxic stress. This phenomenon, recognized as ischemia/reperfusion (I/R) injury, has been a subject of extensive research for over decades.Among the numerous

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mechanisms contributing to I/R injury, oxidative/nitrosative stress, calcium overload, and a pronounced inflammatory response present the major contributors to myocardial damage (Kalogeris et al. 2016). Initial investigations primarily focused on ischemic preconditioning as a strategy for myocardial protection. This approach involves subjecting the heart to brief episodes of I/R before inducing myocardial infarction through prolonged ischemia. Conversely, certain non-ischemic, non-hypoxic stimuli, such as the application of pharmacological agents prior to ischemia, have been explored to mimic the signaling induced by ischemic preconditioning. This exploration aims to alleviate the detrimental effects of I/R injury, providing an alternative avenue for myocardial protection (Li et al. 2015).

Groving evidence suggest that medicinal plants, enriched with polyphenols, harbor the potential to mitigate the detrimental effects of acute myocardial infarction, thereby validating their traditional use in cardiovascular disease prevention. The inhibition of reactive oxygen species (ROS) overproduction through the antioxidant properties found in plant extracts emerges as a significant target for cardioprotection (Bartekova et al. 2010).

Lady's Bedstraw., plant from the Rubiaceae family, has been used in traditional medcicine for the treatment and prevention of various conditions (Lakic et al. 2010). Phytochemical investigation showed that this plant has bioactive compounds such as phenols, iridoid glycosides, anthraquinones, triterpenes, and small quantities of tannins, saponins, and essential oils (Lakic et al. 2010). Although phytochemical investigations have unveiled the plant's chemical composition and confirmed its antioxidant activity in vitro, its biological effects, particularly on cardiac function and oxidative stress, remain partially understood (Vlase et al. 2014).

Considering the presented data, our study aims to evaluate the effects of methanol extract of Lady's Bedstraw on oxidative stress parameters during on ischemia-reperfusion (I/R) injury in isolated rat heart

Materials and methods

Plant material and extract preparation

The whole plant Lady's Bedstraw was collected in the village Dobroselica, on the southern cliff of the Mt Zlatibor. Voucher specimens are deposited in Herbarium of the Institute of Botany and Botanical garden "Jevremovac". The collected material was dried under the shade and powdered (sieve 0.75). Methanol extract was prepared extracting 100 g of aerial part of plant with 500 ml of methanol by heat reflux extraction, at temeperature of 90°C, in a duration of 2 h (Hijazi et al. 2015). The mixture was filtered through filter paper (Whatman, No.1). Dry extract was obtained by evaporation under reduced pressure (RV05 basic IKA, Germany). The residue (17.07 g) was stored in a dark glass bottle at +4°C for further processing. In order to feed the animals, Lady's Bedstraw extract was daily dissolved in the water just before administered to experimental animals.

Animals and experimental design

Twenty Wistar albino rats (males, 8 weeks old, body weight, 200 ± 50 g) were included in the study. The animals consumed commercial rat food (20% protein rat food, Veterinary institute Subotica, Serbia) ad libitum and were housed at temperature of 22 ± 2 °C, with 12 h of automatic illumination daily. The rats were divided into two groups: 1.Control group – rats that drank only tap water; 2. Lady's Bedstraw group – rats that drank tap water containing 500 mg/kg of methanol extract for 28 days.

A day after accomplishing 28-day drinking protocol after ashort-term ket amine/xylazine-induced narcosis, rats were sacrificed by decapitation. The chest was then opened via midline thoracotomy. The hearts were immediately removed and immersed in cold saline and were then attached on a cannula of the *Langendorff* perfusion apparatus to provide retrograde perfusion under constant coronary perfusion pressure of 70 cmH_2O. Following the establishment of heart perfusion, the hearts were stabilised within 30 min. In both groups, after stabilisation period, hearts were subjected to global ischemia (perfusion was totally stopped) for 20 min, followed by 30 min of reperfusion. In the period of reperfusion (30 min), all cardiodynamic parameters and CF were measured in intervals of 5 min (RP1–RP7).

Biochemical analysis in coronary venous effluent and heart tissue

Coronary venous effluent was collected in point of stabilisation (S), in first point of reperfusion (R1) and on every 5 min in period of reperfusion (R1–R7) The following oxidative stress parameters were determined spectrophotometrically (Shimadzu UV 1800, Japan) using collected samples of the coronary venous effluent: the index of lipid peroxidation, measured as thiobarbituric acid-reactive substances (TBARS), nitrite (NO_2-), levels of superoxide anion radical (O_2-) and hydrogen peroxide (H_2O_2).

After accomplishing experiments, hearts from all animals were frozen at -80 °C, and then a 0.5 section of each tissue was homogenised in 5 ml phosphate buffer pH 7.4 using an electrical homogeniser, on ice. Then tissue homogenates were centrifuged at 1200 xg for 20 min at 4 °C. The resulting supernatants were isolated and stored at -80°C until determination of biochemical parameters. Index of lipid peroxidation as well as parameters of antioxidant defence system such as reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) were determined in heart tissue (Bradic J, 2019)

Results and discussion

Biochemical analysis

The index of lipid peroxidation in coronary venous effluent did not differ significantly in the experimental group, while in control group there was a rise in the first minute of reperfusion and at the end of recovery period compared to the values in stabilisation. There was no change in lipid peroxidation in both control and experimental hearts. In the control group, there was an increase in NO_2- production at the first and at the last point of recovery period in comparison to stabilisation. In the group treated with Lady's Bedstraw, higher generation of NO_2- was noticed in the first minute of reperfusion when compared to stabilisation and the end of reperfusion At the end of reperfusion a significantly increased level of O_2- was observed in the control group in comparison to the level before ischemia. On the contrary in Lady's Bedstraw group significantly diminished production of this pro-oxidant was noticed at the end of recovery period compared to stabilisation. The level of H_2O_2 did not vary significantly within the experimental group, while in the control group, enhance in production of H_2O_2 was found at the last point reperfusion in comparison to values before ischemia. The activities of SOD and CAT were significantly higher in the group treated with Lady's Bedstraw in comparison to the control group, while the level of GSH was similar in both observed groups. The analysis of pro-oxidants in the coronary venous effluent provides insights into oxidative stress within the endocardium of the left ventricle and the endothelium of the coronary circulation. Our findings indicated that I/R injury was associated with increased oxidative stress, evidenced by significantly elevated TBARS, NO_2-, O_2^- and H_2O_2 levels in the coronary venous effluent at the end of reperfusion compared to stabilization. However, pretreatment with Lady's Bedstraw led to a reduction in O_2^- levels. Additionally, Lady's Bedstraw extract prevented ischemia-induced lipid peroxidation in heart tissue, thereby preserving membrane integrity.

Table 1. Percent difference in values of oxidative stress parameters between control and Lady's Bedstraw group

Lady's Bedstraw vs control	(%) increase in Stabilisation	(%) increase in RP1	(%) increase in RP7
TBARS	-18.39%	-14.63%	-28.36%
NO_2-	-5.07%	+3.24%	-4.12%
O_2^-	-12.49%	-12.78%	-32.06%
H_2O_2	-5.39%	-11.46%	-22.05%

Concerning the components of the antioxidant defense system, myocardial SOD and CAT activity were higher in the Lady's Bedstraw group, while the level of GSH remained similar between groups. The increased CAT activity might explain the absence of a rise in H_2O_2 production in the Lady's Bedstraw pretreated group, as this enzyme facilitates the decomposition of hydrogen peroxide to water and oxygen. Moreover, the heightened activity of SOD, a key element in antioxidant defense against O_2, aligns with the observed decline in O_2^- levels. It could be hypothesized that the unaltered coronary vasodilatory response, coupled with a decrease in O_2^-, might result from the interplay between increased NO (responsible for the regulation of coronary flow) and the overproduced O_2^-, leading to the generation of toxic peroxynitrite.

Polyphenols, particularly flavonoids, are renowned for their robust antioxidant potential, enabling them to scavenge suddenly generated ROS during the restoration of blood flow. Furthermore, polyphenols activate the endogenous antioxidant defense system, particularly SOD and CAT, leading to a reduction in oxidative stress-induced tissue damage (Mattera et al. 2017). Consequently, the structural and functional integrity of cardiomyocytes is preserved. Moreover, it has been demonstrated that the mechanisms through which polyphenols protect the myocardium from oxidative damage involve the preservation of mitochondrial function, inhibition of xanthine oxidase and nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidase, as well as the chelation of iron ions, which catalyze several free radical-generating reactions (Debnath et al. 2014). The impact on transcription-mediated signaling is responsible for the long-lasting antioxidative effects of these natural molecules (Mattera et al. 2017).

Conclusion

Lady's Bedstraw tended to modulate the activity of myocardial antioxidant enzymes and decrease the generation of pro-oxidants, thus mitigating oxidative stress-induced heart dysfunction.

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BENEFICIAL PROPERTIES OF TRAMETES VERSICOLOR HETEROPOLYSACCHARIDES IN A RAT MODEL OF METABOLIC SYNDROME

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Abstract: We aimed to examine the potentially beneficial effects of Trametes versicolor heteropolysaccharides (TVH) on redox balance in a rats with metabolic syndrome (MetS). Total of 40 Wistar rats were divided into 5 groups: CTRL-healthy non-treated rats; MetS-non-treated rats; H-TV (high dose of TV), M-TV (medium dose of TV) and L-TV (low dose of TV)-rats with MetS treated with either 300, 200 or 100 mg/kg TVH per os for 4 weeks. Prooxidative parameters and parameters of the antioxidative defense system were determined spectrophotometrically. H-TV and M-TV significantly reduced the level of prooxidants and increased antioxidants activity. The obtained results demonstrated that the TVH may be considered a potentially useful agent for redox balance in MetS conditions.

Keywords: Trametes versicolor, heteropolysaccharides, antioxidant, metabolic syndrome

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Introduction

Metabolic syndrome (MetS) can be defined as a disorder including several entities such as insulin resistance, hyperglycemia, hypertension, hyperlipidemia and abdominal obesity according to the WHO (Saklayen, 2018). Over 30 million of people age > 18 have DM2 whereby prevalence of MetS is estimated to be at least three times higher (Smith, 2009). Therapeutic options for MetS management involve changes in lifestyle and the use of antidiabetic and antihyperlipidemic drugs. An alternative medicine approach is also gaining popularity due to several advantages, with significantly fewer side effects being the most important one (Pérez-Martínez, 2017). According to this, more attention has been focused on dietary supplements, nutraceuticals and functional foods used to mitigate hyperglycemia and prevent the development of MetS (Xu, 2018).

Members of the genus *Trametes*, encompassing approximately 60 different species, are highly appreciated species within the mentioned context. *Trametes versicolor (L.) Lloyd*, aka *Coriolus versicolor* or turkey tail mushroom, has been the most thoroughly investigated *Trametes* species so far (Meng, 2022). The *in vitro* antioxidant, anti-inflammatory and antimicrobial effects of T. versicolor have also been reported but there is a lack of data regarding the antidiabetic hypolipidemic properties of this mushroom and its polysaccharides (Bains, 2020).

According to the previous mentioned, we assumed that a 4-week supplementation of TV heteropolysaccharides (TVH) will influence redox status, especially in rats with metabolic syndrome.

Materials and methods

Total of forty healthy male *Wistar albino* rats (six weeks old, body-weight 200 ± 30 g) were included in this study. The animals were fed with standard rat diet (9% fat, 20% protein, 53% starch and 5% fiber), tap water *ad libitum* and housed under controlled regular environmental conditions.

The experimental design was performed in the Center of preclinical and functional research, Faculty of Medical Sciences, University of Kragujevac, Serbia in accordance with the current ethical norms approved by the Ethics Committee of the Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia, number: 06/17.

After a one-week environment adaptation, MetS was induced by feeding the rats with high-fat diet (HFD—25% fat, 15% protein, 51% starch and 5% fiber) for 4 weeks followed by a single intraperitoneal injection of streptozotocin (STZ) in a dose of 25 mg/kg (Abdel-Hamid, 2019). MetS was confirmed by measuring fasting glucose and insulin levels, lipid status and blood pressure 72 h post-streptozotocin injection and the animals with fasting blood glucose.

All rats were divided into the five groups: CTRL (n = 8)—untreated healthy animals fed with standard diet; MetS (n = 8)—untreated rats with induced MetS; H-TV (n = 8)—rats treated with 300 mg/kg (high dose of TV); M-TV (n = 8)—rats treated with 200 mg/kg (medium dose of TV); L-TV (n = 8)—rats treated with 100 mg/kg (low dose of TV). Groups of animals with induced MetS were fed with HFD till the end of the study. TVH was administered per os at the appropriate dose every day at the same time for four weeks.

All animals were sacrificed, and blood samples were collected from jugular vein to evaluate the systemic redox state. Blood samples were centrifuged in order to separate the plasma and red blood cells, which were stored at −20 °C until biochemical analysis. The following pro-oxidant parameters were determined from plasma samples: hydrogen peroxide (H_2O_2), superoxide anion radical (O_2^-), nitrites (NO_2^-), and index of lipid peroxidation (TBARS). Parameters of the antioxidative defense system were determined from erythrocyte lysate samples: the activity of superoxide dismutase (SOD) and catalase (CAT) and the level of reduced glutathione (GSH) as previously described (Jeremic, 2018).

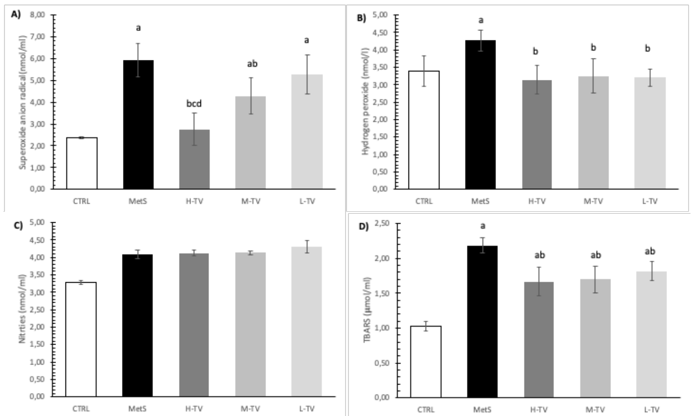
Results and discussion

The induction of MetS led to a significant increase in the release of prooxidants, O_2^-, H_2O_2 and TBARS, compared to healthy CTRL rats ($p < 0.05$). The treatment with TVH significantly decreased the release of all measured prooxidant parameters, O_2^-, H_2O_2 and TBARS, except NO_2^-. The H-TV group had the most prominent effect on O_2^- compared to medium and low doses of TVH supplementation ($p < 0.05$).

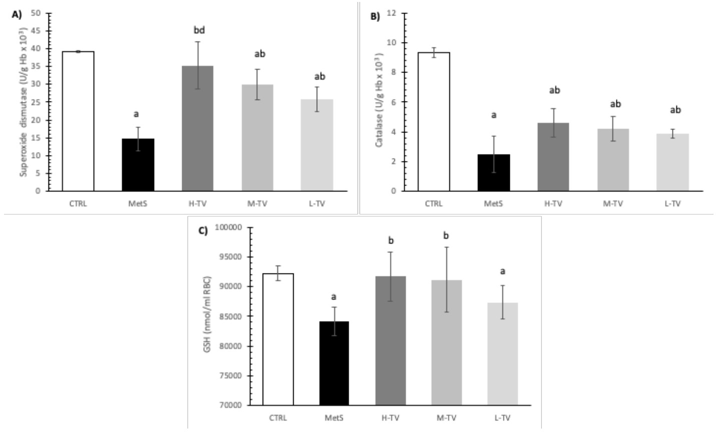
The rats with MetS showed signs of highly compromised antioxidant systems; their levels of SOD, CAT and GSH were significantly lower than in control group of healthy individuals ($p < 0.05$).

The four-week administration of TVH in all three investigated doses induced a significant increment of antioxidant enzymes activity (SOD and CAT) compared to the MetS rats. The increase in SOD activity in the H-TV group was superior relative to the L-TV group ($p < 0.05$), while there were no significant differences in CAT activity between the three applied doses of TV ($p > 0.05$; Figure 3A,B).

These results correlate with previuos findings, suggesting that the amelioration of oxidative stress by TVH in MetS conditions may also originate from polysaccharopeptides, besides the phenolic acids and flavonoids mentioned (Lo, 2020).



Graph 1. Effects of TVH administration on antioxidant parameters. (A) Superoxide anion radical (O_2^-), (B) hydrogen peroxide (H_2O_2); (C) nitrites (NO_2^-); (D) index of lipid peroxidation (TBARS). CTRL: control group of healthy non-treated rats; MetS: control group of rats with induced metabolic syndrome; H-TV: rats with metabolic syndrome treated with high dose of T. versicolor; M-TV: rats with metabolic syndrome treated with medium dose of T. versicolor; L-TV: rats with metabolic syndrome treated with low dose of T. versicolor. Data are presented as means ± standard deviation. Statistical significance at the level $p < 0.05$: a compared to CTRL; b compared to MetS; c compared to HTV group; d compared to M-TV group.



Graph 2. Effects of TVH administration on antioxidant parameters. (A) Superoxide dismutase (SOD), (B) catalase (CAT) and (C) reduced glutathione (GSH). CTRL: control group of healthy nontreated rats; MetS: control group of rats with induced metabolic syndrome; H-TV: rats with metabolic syndrome treated with high dose of T. versicolor; M-TV: rats with metabolic syndrome treated with medium dose of T. versicolor; L-TV: rats with metabolic syndrome treated with low dose of T. versicolor. Data are presented as means ± standard deviation. Statistical significance at the level p <0.05: a compared to CTRL; b compared to MetS; d compared to L-TV group.

Conclusion

H-TV and M-TV significantly reduced the level of prooxidants (O_2^-, H_2O_2, TBARS; $p < 0.05$), increased antioxidants activity (SOD, CAT, GSH; $p < 0.05$). The obtained results demonstrated that the TVH may be considered a potentially useful agent for redox balance in MetS conditions.

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ASSESSMENT OF *GALIUM VERUM* EXTRACT SUPPLEMENTATION ON REDOX HOMEOSTASIS IN PSORIATIC RAT MODEL

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Abstract

Psoriasis lacks comprehensive data on its systemic oxidative impact. This study explores *Galium verum* extract's influence on systemic oxidative state in psoriatic rats. Twenty-four male W.albino rats were divided into control (CTRL), psoriasis (PSORI) and psoriasis treated with *G.verum* extract (PSORI+GV) groups. Blood samples analyzed for redox state biomarkers revealed increased TBARS levels in PSORI *vs* CTRL and PSORI+GV. PSORI exhibited significantly lower nitrite levels compared to CTRL and PSORI+GV, with elevated O_2^- and H_2O_2 levels. GSH and SOD values were reduced in PSORI and PSORI+GV, while catalase activity increased. *G.verum* extract positively modulated psoriatic rat redox state.

Keywords: psoriasis, redox homeostasis, *Galium verum* extract

Introduction

Psoriasis, a chronic inflammatory skin disorder characterized by dysregulated immune responses and aberrant keratinocyte proliferation, poses significant challenges in understanding its underlying mechanisms. Psoriasis is characterized by sharply demarcated erythematous skin lesions adorned with silver hyperkeratotic plaques, often accompanied by systemic manifestations. Its prevalence varies across diverse populations and geographical regions globally. Estimates indicate that approximately 2-3% of the world's population

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is afflicted by psoriasis. Nevertheless, prevalence rates can escalate in specific regions, ethnicities, and age cohorts (Medovic et al., 2022).

Among the various factors implicated, oxidative stress stands out for its role in perpetuating inflammation and tissue damage in psoriatic lesions. Oxidative stress, arising from an imbalance between reactive oxygen species (ROS) or reactive nitrogen species (RNS) production and antioxidant defense mechanisms, plays a critical role in the pathogenesis of psoriasis. This imbalance may arise from reduced activity of antioxidative enzymes, including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx), or a decrease in the level of scavenging antioxidants, both endogenous (e.g. reduced glutathione (GSH)) and exogenous (such as vitamin C, vitamin E, and carotenoids). At low concentrations, ROS/RNS serve as signaling molecules in the regulatory cascades of various biological processes. However, excess ROS/RNS and consequent oxidative stress can induce oxidation of cellular structures, including deoxyribonucleic acid (DNA), lipids, and proteins, ultimately leading to cell death. The skin, being constantly exposed to environmental stressors, is particularly vulnerable to oxidative damage. In psoriasis, this delicate balance is disrupted, leading to sustained oxidative stress within the cutaneous microenvironment. So the redox imbalance in psoriasis has been reported in skin cells and, systemically, in plasma and blood cells as well (Ambrozewicz et al., 2018).

Numerous treatments, including topical medications with corticosteroids, retinoids, vitamin D_3 analogues, anthralin, and systemic medications with diverse mechanisms of action, are used for psoriasis. However, most therapies are unsuitable for long-term use due to serious side effects and high costs. Commonly used drugs like methotrexate, acitretin, cyclosporine, dexamethasone, and salicylic acid have limited efficacy and pose challenges such as low absorption, inconsistent release, and adverse reactions (Lai et al., 2018).

Throughout history, traditional medicine has utilized medicinal plants for therapeutic purposes. A multitude of plant species possess inherent anti-inflammatory and antioxidant attributes, among which *Galium verum* (GV) stands notable (Goncalves et al., 2023). *Galium verum* L., a perennial herbaceous plant from the *Rubiaceae* family, boasts a rich historical legacy of employment in treating and preventing various ailments. This plant species has yielded diverse classes of bioactive compounds, including phenols, triterpenes, iridoid glycosides, and anthraquinones, alongside trace amounts of saponins, tannins, and essential oils. *Galium verum* extract, derived from the plant *Galium verum* L., has attracted attention for its antioxidant properties and potential therapeutic effects in inflammatory conditions. Its significance in traditional medicine dates back to ancient times, esteemed for its multifaceted therapeutic properties such as sedative, spasmolytic, anticancer, and cardioprotective effects (Turcov et al., 2022). Studies have also demonstrated its ability to scavenge free radicals, reduce oxidative damage, and modulate inflammatory pathways (Bradic et al., 2020). However, as far as we know based on the available literature, there have been no investigations of psoriasis in an animal model with the therapeutic application of *Galium verum* extract. Therefore, this study aims to elucidate the impact of *Galium verum* extract administration on systemic redox balance in psoriasis-induced rats.

Material and methods

Experimental animals

A total of 24 adult male Wistar albino rats, aged 12 weeks with body weight (BW) of 250-300 g were included in the study. The animals were randomly divided into three groups (n=8 in each group) and were housed in separate clean plastic cages under controlled standard environmental conditions of temperature (22 ± 1 °C) and light (12/12 h light/dark cycle). Food and water were provided ad libitum throughout the treatment.

Treatment protocol and experimental design

After a week of adaptation to the controlled conditions, the rats were divided into three equal groups: control (CTRL), psoriatic (PSORI), and psoriatic rats treated with *Galium verum* extract (PSORI+GV). CTRL group were healthy rats, while the PSORI group consisted of rats in which psoriasis was induced according to the protocol described below. The PSORI+GV group consisted of rats with induced psoriasis that were treated with daily oral administration of methanol extract of *Galium verum* at a dose of 250 mg/kg BW for a total of 4 weeks. The methanol extract of *Galium verum* was prepared according to the protocol for extract preparation described in the literature (Bradic et al., 2020).

Induction of psoriasis

Psoriasis was induced using 5% imiquimod cream, an agonist of toll-like receptors (TLR) 7/8 of macrophages, monocytes, and dendritic cells. TLR 7/8 is known for its potent immunoactivating properties and induction of apoptosis. The cream was applied topically on the shaved back skin of the rats (3 cm x 2.5 cm) once daily for seven consecutive days. Imiquimod cream was selected for psoriasis induction due to its ability to locally induce psoriatic dermatitis, resembling the morphological and clinical characteristics of psoriasis. Despite its clinical indications for other conditions, imiquimod has been observed to induce worsening of psoriasis and the development of new lesions even in well-controlled patients. This model offers a straightforward approach for psoriasis induction, making it valuable for preclinical studies aimed at investigating pathophysiological and cellular mechanisms underlying psoriasis (Chatterjee and Sur, 2024).

Sample collection

After completion of the experimental protocol, the rats were sacrificed, and blood samples were collected. Plasma and erythrocytes were isolated from the blood samples for the determination of oxidative stress parameters using spectrophotometric method (Shimadzu UV 1800, Japan).

Determination of oxidative stress biomarkers

Determination of the index of lipid peroxidation (measured as TBARS)

The concentration of lipid peroxidation in the plasma was assessed by measuring TBARS using 1% thiobarbituric acid in 0.05 NaOH, incubated with 0.8 mL of plasma samples at 100 °C for 15 min and measured at 530 nm (Ohkawa et al., 1979).

Hydrogen peroxide (H_2O_2) determination

The assessment of H_2O_2 was performed using the oxidation of phenol red with H_2O_2 in a reaction catalyzed by horseradish peroxidase (HRPO). A 200 µl plasma sample was precipitated with 800 µl of phenol red solution with 10 µl of (1:20) HRPO added. An adequate volume of Krebs–Henseleit solution was used as a blank. The degree of H_2O_2 was measured at 610 nm (Pick and Keisari, 1980).

Superoxide anion radical (O_2^-) determination

The degree of O_2^- was measured by the reaction of nitro blue tetrazolium in 2-amino-2-(hydroxymethyl)-1,3-propanediol with plasma samples at 530 nm. Distilled water was used as a blank probe (Auclair and Voisin, 1985).

Nitrite (NO_2^-) determination

The level of NO_2^- was determined as an index of nitric oxide production. A 0.5 mL plasma sample was precipitated with 200 µl of 30% sulfosalicylic acid, vortexed, and then centrifuged at 3000g. The resulting supernatant was mixed with an equal volume of Griess reagent, incubated for 10 min in the dark, and measured at 550 nm. The NO_2^-concentrations were calculated using sodium nitrite as the standard (Green et al., 1982).

Determination of CAT activity

The activity of CAT was estimated according to Aebi (1984). Lysates of erythrocytes were diluted in distilled water and treated with chloroform-ethanol to eliminate hemoglobin. Then, 50 μl of CAT buffer, 100 μl of sample, and 1 mL of 10 mM H_2O_2 were added to the samples. Measurement was performed at 360 nm.

Determination of SOD activity

SOD activity was determined by the epinephrine method of Misra and Fridovich (1972). A total of 100 μl lysate of erythrocytes and 1 mL of carbonate buffer were mixed, then 100 μl of epinephrine was added and detected at 470 nm.

Reduced glutathione (GSH) determination

The level of GSH was assessed based on GSH oxidation with 5,5-dithio-bis-6,2-nitrobenzoic acid using the method of Beutler (1975). Measurement was made at 412 nm.

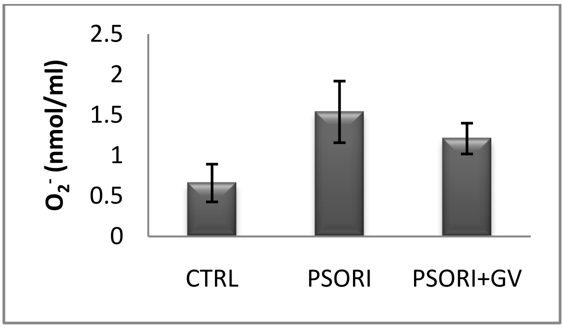
Statistical analysis

Data are presented as the mean ± SD. The effects on oxidative parameters and antioxidant status between groups were analysed by one-way ANOVA (Scheffe's F test) and the nonparametric analogue test of ANOVA (Kruskall–Wallis test). Differences were considered to be significant at $p< 0.05$. Statistical analysis was performed with SPSS version 24.0 statistical package (IBM SPSS Statistics 24).

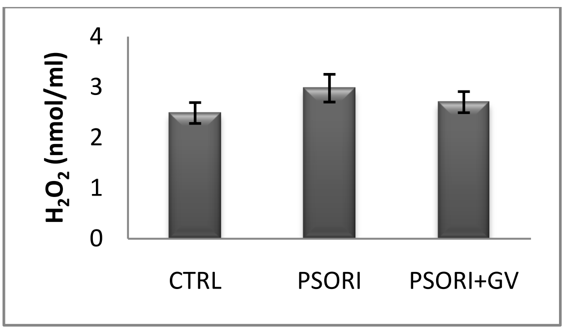
Results and discussion

The effects of *Galium verum* extract on pro-oxidative parameters (TBARS, H_2O_2, O_2^-,NO_2^-) in plasma

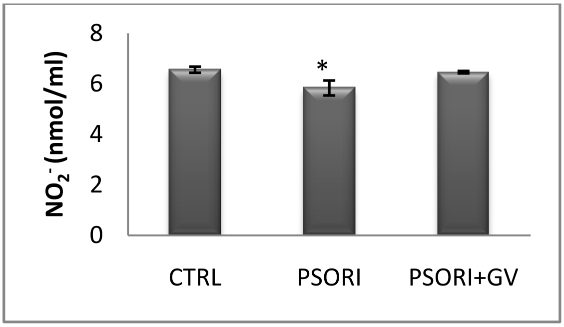
Our results showed that the concentrations of TBARS, H_2O_2 and O_2^- were significantly elevated in PSORI group compared to CTRL and PSORI+GV as well. On the other hand, the level of nitrites in plasma were decreased in rats with psoriasis in comparison with control and PSORI+GV group (Graphs 1-4). Since this is the first such research and there are no investigations with psoriatic animal model and treatment with *Galium verum* extract, we can only interpret our results in the light of the application of this plant extract on models of other diseases and analysis of oxidative stress. Two such studies referred to the evaluation of effects of *Galium verum* extract on the functional recovery and redox equilibrium of isolated rat heart after ischemia. The levels of nitrites and superoxide anion radical observed in our investigation align with the findings of Bradic et al. (2019, 2020), who delineated a similar pattern in pro-oxidative parameters within cardiac tissue subsequent to ischemia-reperfusion injury. This concurrence points to a presumed cardioprotective effect of *Galium verum* extract, which acts by modulating the redox balance and mitigating the generation of free radicals, similar to our model of psoriasis in rats where this extract positively modulates systemic redox homeostasis.



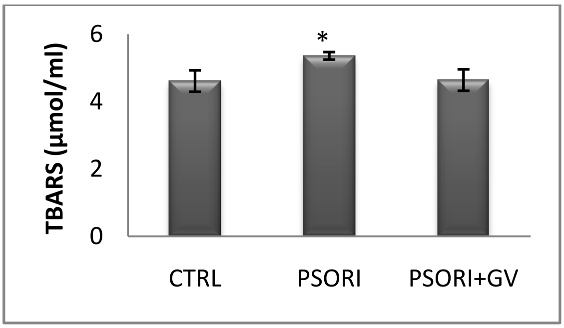
Graph 1. The level of O_2^- in plasma



Graph 2. The level of H_2O_2 in plasma



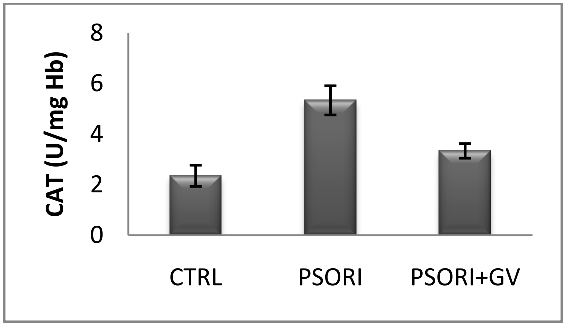
Graph 3. The level of NO_2^- in plasma



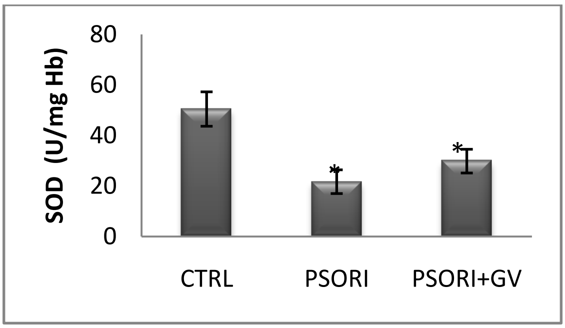
Graph 4. The level of TBARS in plasma

The effects of *Galium verum* extract on antioxidative parameters (CAT, SOD, GSH) in erythrocytes

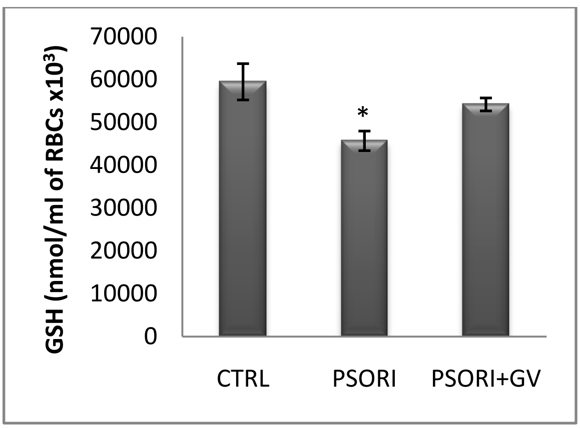
In our study the activity of antioxidative enzyme catalase was increased in both rats with psoriasis and those that was treated with *Galium verum* extract (Graph 5). The level of reduced GSH as well as the activity of SOD enzyme were decreased in PSORI and PSORI+GV groups compared to CTRL group (Graphs 6, 7). The dynamics of antioxidant defense enzyme activity correspond to the observed trends in these parameters within the myocardial tissue of rats subjected to ischemia/reperfusion injury, yet diverge from the values documented in the myocardial tissue of spontaneously hypertensive rats under identical ischemic injury conditions (Bradic et al., 2019, 2020).



Graph 5. The activity of CAT in erythrocytes



Graph 6. The activity of SOD in erythrocytes



Graph 7. The level of GSH in erythrocytes

Conclusion

This study aimed to provide a comprehensive overview of the intricate interaction between oxidative stress and the pathogenesis of psoriasis, while also investigating the therapeutic potential role of *Galium verum* extract in alleviating oxidative stress and ameliorating the clinical manifestations of psoriasis. Although this is the pioneering investigation in employing a psoriatic animal model treated with *Galium verum* extract, our findings suggest potential therapeutic implications as it shows a beneficial effect on redox balance modulation in psoriasis. Moreover, the potential synergistic effects of *Galium verum* extract with conventional treatments and its safety profile warrant further investigation.

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THE EFFECTS OF CHRONIC ADMINISTRATION OF *ALLIUM URSINUM* EXTRACT ON SYSTEMIC REDOX STATUS IN RATS

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Abstract: The present study aimed to estimate the effects of chronic treatment with *Allium ursinum* extract on the redox status in rats. The administration of extract in a dose of 500 mg/kg significantly reduced the concentration of all tested pro-oxidative markers compared to the control group. Additionally, the application of extract caused a significant elevation in the antioxidant activity compared to the control group. Our results have shown that the four-week intake of the methanolic *Allium ursinum* extract significantly modulates the redox status and stands out as a powerful antioxidant.

Keywords: *Allium ursinum*, extract, oxidative stress, rat

Introduction

Allium ursinum L. (synonyms: wild garlic, ramson, or bear garlic) is a perennial herbaceous plant belonging to the Alliacee family and has been used for centuries in traditional medicine as a prophylactic and therapeutic agent. Several classes of bioactive compounds have been isolated from A. ursinum so far, among them sulfur and phenolic compounds as quantitatively the most distributed constituents, responsible for the pharmaceutical effects of the plant (Sobolewska et al., 2015). Taken into consideration that oxidative stress response to *A. ursinum* has still not been clarified, the aim of this study was to assess the effects of this extract on systemic redox status in male *Wistar albino* rats.

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Materials and methods

Plant Material and Extract Preparation

The whole plant *A. ursinnum* was collected in May on Mt Bukulja. The collected material was dried under the shade and powdered (sieve 0.75). The methanol extract was prepared by extracting 100 g of aerial part of the plant with 500 ml of methanol by heat reflux extraction, at the temperature of 90°C, in 2 h (Hijazi et al., 2015). The mixture was filtered through filter paper. The dry extract was obtained by evaporation under reduced pressure. The residue was stored in a dark glass bottle at +4°C for further processing. To feed the animals, AUE was daily dissolved in the water just before administered to experimental animals.

Animals and Experimental Design

The present study was carried out on 40 male *Wistar albino* rats. Rats were randomly divided into four groups depending on the applied treatment.

1. CTRL, rats who drank only tap water for 28 days;
2. 125 AUE, rats who drank tap water containing 125 mg/kg of methanol extract of *A. ursinum* for 28 days;
3. 250 AUE, rats who drank tap water containing 250 mg/kg of methanol extract of *A. ursinum* for 28 days;
4. 500 AUE, rats who drank tap water containing 500 mg/kg of methanol extract of *A. ursinum* for 28 days.

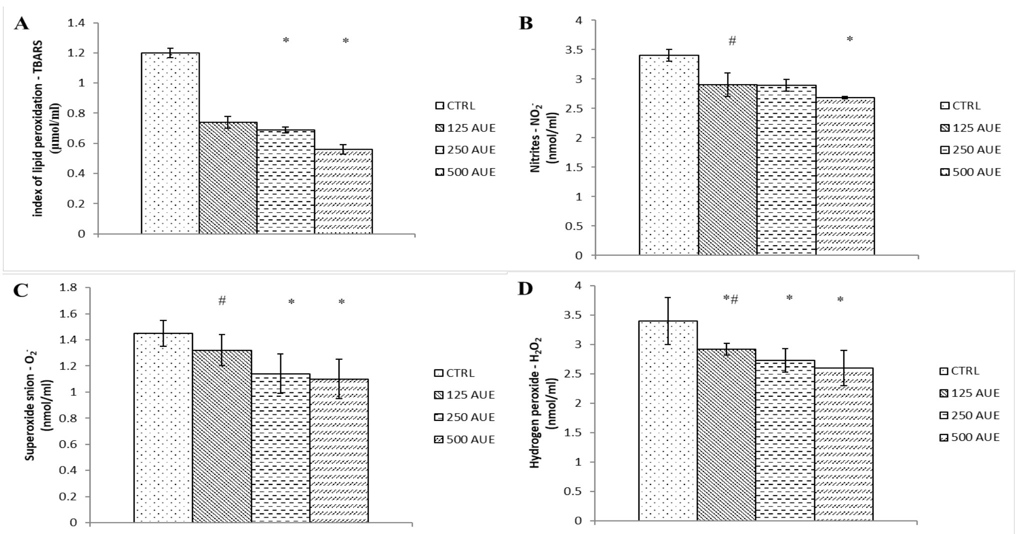
Oxidative Stress Markers and Antioxidant Enzyme Determination

After four weeks, the rats were sacrificed and the blood samples were collected from a jugular vein to test the systemic redox state. Plasma samples and erythrocytes were separated via centrifugation of heparinized venous blood. In plasma samples, we measured the concentration of pro-oxidative markers such as the index of lipid peroxidation, measured as thiobarbituric acid-reactive substances (TBARS), nitrites (NO_2^-), superoxide anion radical (O_2^-), and hydrogen peroxide (H_2O_2). In the lysate, we determined the activity of non-enzymatic antioxidants, including reduced glutathione (GSH) and the activity of the enzymatic defense system by evaluating the catalase (CAT) and superoxide dismutase (SOD) levels (Bradic et al., 2019). All mentioned biochemical parameters of oxidative stress were determined spectrophotometrically (Shimadzu UV-1800UV-VIS spectrophotometer, Japan).

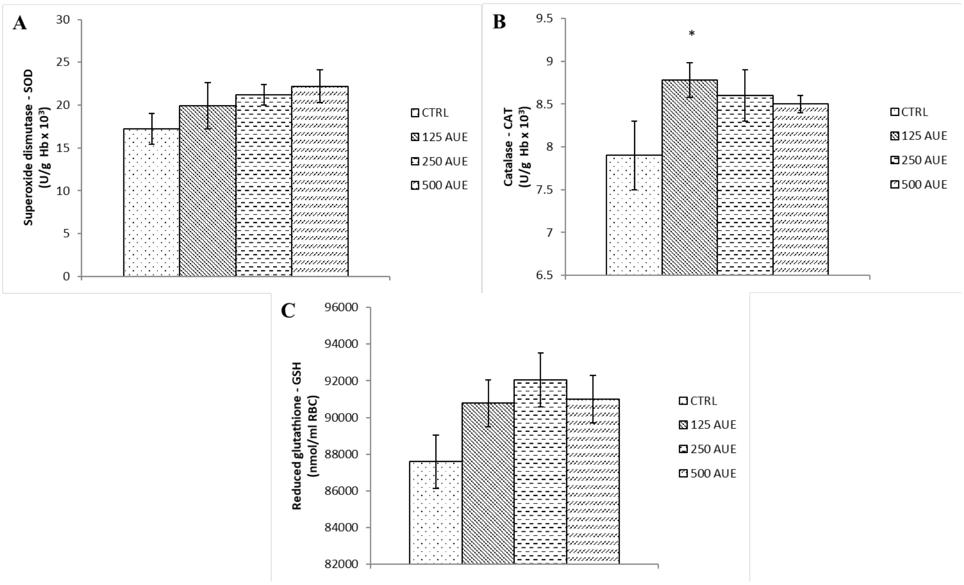
Results and discussion

All tested pro-oxidants were statistically significantly reduced in the group of rats using AUE at a dose of 500 mg/kg compared to the CTRL group. Application of the extract at a dose of 250 mg/kg reduced the values of O_2^-, H_2O_2, and TBARS, while the lowest dose led to a decrease in the concentration of TBARS and H_2O_2 compared to untreated rats. By comparing the values of pro-oxidants between the groups on the treatment with different doses, lower values of all markers are observed after the treatment with the extract at a dose of 500 mg/kg in relation to 125 mg/kg (Graph 1.).

The application of AUE caused a significant elevation in the antioxidant activity compared to CTRL. However, the highest dose of extract managed to lead to the highest SOD value compared to the control group, while the application in the lowest dose led to a significant jump in the CAT value. On the other hand, the GSH activity did not change significantly under the influence of AUE in all three doses (Graph 2).



Graph 1. Effects of consumption of increasing doses of AUE on the value of pro-oxidative markers in plasma samples: TBARS (A), NO_2^- (B), O_2^- (C), H_2O_2 (D). Values are shown as mean ± SE. *Statistically significant difference at the level of $p < 0.05$ in relation to the CTRL group; #Statistically significant difference at the level of $p < 0.05$ compared to 500AUE.



Graph 2. Effects of consumption of increasing doses of AUE on the value of SOD (A), CAT (B), and GSH (C). Values are shown as mean ± SE. *Statistically significant difference at the level of $p < 0.05$ in relation to the CTRL group

The results demonstrated a significant drop in the values of all markers after treatment with the extract compared to the CTRL group but with the most prominent changes in groups treated with the highest dose. The same trend was observed in SOD activity, while when it comes to CAT activity, the lowest dose led to the most significant increase in relation to the CTRL group. However, GSH levels did not change significantly after 4 weeks of intake of *A. ursinum* in all three doses. In a study by Masjedi et al. (2013), methanol extract of *A. ursinum* was applied in diabetic rats at doses of 60 and 120 mg/kg. The activity of SOD and CAT was significantly increased in treated animals, which is in accordance with the results, providing the ability of *A. ursinum* to reduce oxidative stress and provide beneficial systemic effects. A previously conducted study examined whether *A. ursinum* has better antioxidant properties compared to other *Allium* species. It was observed that wild garlic possesses a relatively higher concentration of antioxidants and better scavenger activity compared to the other *Allium* species from Balkan flora. The results of this study clearly indicated the ability of *Allium* species to remove free radicals and prevent oxidative stress from occurring (Stajner et al., 2008). According to a previously published study, methanolic extract was considered to be the richest in the number of phenolic acids and flavonoids due to its strongest ability (followed by ethanol) to isolate phenolic compounds (Pavlović et al., 2017). Contrastingly, the results of the study conducted on cell lines that are previously exposed to doxorubicin treatment indicate the powerful antioxidant effect of ethanol AUE but poor the effect of the methanolic extract on the antioxidant capacity of wild garlic. The discrepancy between their results and the findings of other studies may be attributed to different study designs and measurement of the impact of *A. ursinum* on different parameters of redox status by using various tests (Pop et al., 2020).

Conclusion

In summary, our results pointed out that a four-week administration of *A. ursinum* extract leads to beneficial adaptations, manifested as decreased oxidative stress and an improved antioxidant defense system. In addition, these findings can lead to the development of strategies to prevent oxidative stress. Further investigations should be conducted to clarify the exact mechanism of the *A. ursinum* extract protective effects.

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EFFECTS OF POMEGRANATE JUICE ON LIPID METABOLISM IN WOMEN WITH DYSLIPIDEMIA AND METABOLIC SYNDROME

Aleksandra Arsić, Milica Kojadinović, Snjezana Petrović, Danijela Ristić Medić, Milena Žuža Praštalo, Vesna Vučić

Abstract: The objective of this research was to evaluate the effects of the consumption of Pomegranate juice (PJ) on blood pressure and lipid metabolism in subjects with dyslipidemia and metabolic syndrome. Twelve females with established dyslipidemia and 12 females with metabolic syndrome consumed 300 mL of PJ daily for 2 and 6 weeks respectively. PJ consumption resulted in a significant decrease in diastolic blood pressure, and LDL-cholesterol, an increase in the estimated activity of stearoyl-CoA desaturase in the short study, and a significant decrease in the percentage of arachidonic acid and an increase in monounsaturated fatty acids in longer study. These results indicate a positive impact of the consumption of pomegranate juice, in both short and long periods, on lipid metabolism and suggest potential anti-inflammatory and cardio-protective effects.

Keywords: Pomegranate juice, Dyslipidemia, Metabolic syndrome, Lipid profile, Arachidonic acid

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POLYPHENOL-RICH BLACK CURRANT JUICE AMELIORATES METABOLIC SYNDROME INDUCED BY HIGH-FAT HIGH-FRUCTOSE DIET IN RATS

Snježana Petrović[1], Marija Paunović[1], Nataša Veličković[2], Bojana Micić[2], Maja Milošević[3], Aleksandra Arsić[1], Biljana Pokimica[1], Vesna Vučić[1]

Abstract: Our study was aimed to investigate the effects of commercially available, cold-pressed polyphenol-rich black currant (BC) juice on fat accumulation, blood lipids, insulin resistance, high blood pressure, and inflammation, in rats placed on high-fat high-fructose (HFF) diet. Co-administration of BC juice significantly reduced diet-induced adiposity and plasma triglycerides levels, improved glucose tolerance, and suppressed hepatic inflammation during prolonged HFF feeding in rats. These findings suggest that consumption of BC juice may counter the harmful effects of the Western diet rich in fats and sugars, and to be useful for the prevention of metabolic syndrome development and progression.

Keywords: polyphenol-rich juices, black currant, high-fat high-fructose diet, metabolic syndrome, *Wistar* rats

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