TECHNOLOGICA ACTA

SCIENTIFIC/PROFESSIONAL JOURNAL
OF CHEMISTRY AND TECHNOLOGY
FACULTY OF TECHNOLOGY
UNIVERSITY OF TUZLA

ISSN 1840-0426 (P)
ISSN 2232-7568 (E)
DOI: 10.51558/2232-7568

Vol. 17
No. 1
Pages 1-80
October 2024
Tuzla, Bosnia and Herzegovina

FACULTY OF TECHNOLOGY
UNIVERSITY OF TUZLA

65TH ANNIVERSARY
1959-2024

https://hrcak.srce.hr/ojs/index.php/technologicaacta
http://tf.untz.ba/technologica-acta

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Vol. 17, No. 1, October 2024 Pages 1-80

Publisher	Faculty of Technology, University in Tuzla
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Technical Editing & Layout	Franc Andrejaš
Cover Illustration	Artwork
Editorial Office	Technologica Acta Faculty of Technology, University in Tuzla Urfeta Vejzagića 8, 75000 Tuzla, Bosnia and Herzegovina Phone/fax: +387 35 320 754 / +387 35 320 741 E-mail: franc.andrejas@untz.ba
Printing	IN SCAN d.o.o. Tuzla
Circulation	100
Available online at:	https://hrcak.srce.hr/ojs/index.php/technologicaacta http://tf.untz.ba/technologica-acta
Technologica Acta is indexed in	CAB Abstracts, COBISS, Index Copernicus Journal Master List, EBSCO, HRČAK, ROAD, Directory of Journal Quality Factor, Publons, Norwegian Register for Scientific Journals, Series and Publishers... Also recognized by Google Scholar, ResearchGate, OpenAIRE etc.

Technologica Acta is published twice a year.

TECHNOLOGICA ACTA
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CONTENT


UNIVERSITAS STUDIORUM TUZLAENSIS
TEHNOLOŠKI FAKULTET
1959
TUZLA
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A VIGNETTE ON THE 65TH ANNIVERSARY OF THE FACULTY OF TECHNOLOGY AND THE DEAN'S ADDRESS TO THE 2024/2025 FRESHMAN CLASS

EDITORIAL

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DOI: 10.51558/2232-7568.2024.17.1.1

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ABSTRACT:
A Vignette on the 65th Anniversary of the Faculty of Technology and the Dean's Address to the 2024/2025 Freshman Class

The Faculty of Technology in Tuzla is the oldest higher education institution in the Tuzla Canton and one of the oldest in Bosnia and Herzegovina. It was established in 1959 and was the cornerstone for the founding of the University of Tuzla.

This year, the Faculty of Technology celebrates 65 years of existence and work, marked by continuous development and success. The faculty has played a significant role in the education of students in Bosnia and Herzegovina and beyond, as well as in the founding of other higher education and scientific institutions in Tuzla, which eventually led to the establishment of the University of Tuzla. The Faculty of Technology in Tuzla was the first higher education institution in Bosnia and Herzegovina to initiate postgraduate studies in 1963. The first two master's theses were defended in 1965, and the first doctoral dissertation was defended in 1962.

Today, the Faculty has a total area of 7,878 square meters, comprising modernly equipped amphitheaters, classrooms, laboratories, a library, and faculty offices. Long-standing practice and constant collaboration with the industry have led to the development of curricula that provide the opportunity for training and educating engineers who have sufficiently broad knowledge to quickly adapt to the demands of the industry. Teaching is organized across all three cycles of studies, within the following study programs:

First Cycle of Studies
- Chemical Engineering and Technology
- Food Technology
- Environmental Engineering
- Agronomy

Second Cycle of Studies
- Chemical Engineering and Technology
- Food Technology
- Environmental Engineering
- Agronomy

Third Cycle of Studies
- Chemical Engineering
- Environmental Engineering
- Food Engineering
- Applied Chemistry (joint doctoral program of the Faculty of Technology and the Faculty of Science and Mathematics)

The faculty's strategy in the field of education involves an optimal balance between European values and local traditions in educating students, who are the foundation of society's future and the greatest inspiration for teachers to effectively impart their knowledge and prepare them for the professions they have chosen. In this regard, the Dean of the Faculty, Prof. Dr. Sci. Sead Ćatić, traditionally delivered a speech addressing the 65th generation of freshmen at the Faculty of Technology, University of Tuzla:

„Dear Vice Deans, Heads of Departments, Professors, Assistants, and other staff, and most importantly, dear students,

It is my pleasure and honor to warmly welcome all of you on my behalf and on behalf of all the employees of the Faculty of Technology. I would like to thank you for being here and for joining us today to celebrate the beginning of the academic year 2024/2025. Welcome to the academic community and the Faculty of Technology.

I would like to remind you of an important fact that sets us apart among the 12 faculties and one academy at the University of Tuzla, which is that the Faculty of Technology was the first to be established, specifically in 1959, when the first generation of students was enrolled. Because of this, the Faculty of Technology has the longest tradition and the most experience in higher education in the Tuzla Canton. The history of our faculty is filled with successes, innovations, and dedicated efforts to enhance the knowledge and skills of our students. Since its founding, the Faculty has produced many excellent engineers who have held, or continue to hold, responsible positions; 4,000 graduates, 300 master's degree holders, and 95 doctoral degree holders are indicators of the long and rich history of the Faculty of Technology.

The decades-long existence and successful operation of this institution have enabled us to develop strong collaborations with other higher education institutions, both domestically and internationally. These collaborations allow our students to participate in international projects, exchange knowledge with students from other related faculties, and gain global perspectives in their fields. In addition to academic partnerships, the Faculty of Technology has established strong connections with business and industrial enterprises in the surrounding areas and beyond. Such connections provide our students with a unique opportunity for practical experience, working on real projects, and preparing for a successful career. Our graduates are recognized as highly qualified professionals, ready to face the challenges of the modern job market.

Today is an important day for the Faculty of Technology! A day when we welcome you, the youth, as the 65th generation that will continue the rich tradition of our, and from today, your faculty. You will spend the next four years acquiring knowledge and skills, exchanging ideas, and gaining your first serious life experiences. Because of this fact, we all feel a sense of responsibility—toward society, the state, and your families. Our esteemed professors and researchers are leading experts in their fields and are here to guide and inspire you. During your studies, you will have access to laboratories and research equipment, as well as opportunities to participate in significant research projects and recognized scientific and professional conferences.

At Faculty of Technology, the emphasis is on innovation, critical thinking, and solving practical problems. We encourage you to be curious, to explore, to ask questions, and to seek answers. Your success is our priority, and the entire team of professors and staff is here to support you on that journey. As you adapt to your studies, we urge you to make the most of the diverse opportunities available to you. Get involved in student organizations, attend conferences, seminars, and workshops, and collaborate with your peers. Participating in extracurricular activities and building a strong network of acquaintances with students from this and other faculties will enrich your educational experience and prepare you for a successful career.

Remember, the journey you have just begun will not always be easy. There will be challenges and obstacles along the way. But it is through overcoming these challenges that you will grow and develop as professionals and as individuals. Do not hesitate to seek support from your professors, mentors, and fellow students. We are all here to help you succeed.

Dear students, I take this opportunity to congratulate you on the beginning of an important chapter in your life, a chapter in which you will acquire the knowledge and skills that you will later apply in practice at your future workplaces. I would also like to extend my sincere congratulations to the professors and staff who have contributed to the growth and development of the Faculty of Technology over the years, who represent this faculty in the best possible way, and who support your development. I am confident that you, as representatives of this institution, will continue to follow the paths paved by generations of students from the Faculty of Technology at the University of Tuzla before you. In addition to the diploma you will receive upon completing your studies, carry with you the knowledge you have gained. May you bring joy to your parents with your successes, as well as to us.

In conclusion, I would like to remind you that the fields of Chemical Engineering and Technology, Environmental Engineering, Food Technology, and Agronomy are among the most dynamic and influential areas of study. The knowledge and skills you acquire here will enable you to make a significant contribution to society, whether through improving existing industrial processes, developing food or chemical products and materials, or enhancing environmental protection.

Once again, congratulations on your enrollment at the Faculty of Technology. We are proud to have you with us and look forward to all your achievements in the coming years.

Welcome to the Faculty of Technology—I wish you a successful start to your studies, happiness, and good health.*"*

THE CONTENT OF HEAVY METALS IN HONEY AS INDICATORS OF POLLUTANTS

ORIGINAL SCIENTIFIC PAPER

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DOI: 10.51558/2232-7568.2023.17.1.3

RECEIVED 2023-10-10 **ACCEPTED** 2024-07-08

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ABSTRACT:
Honey and honeydew are natural foods with a very complex composition that contain both, organic and inorganic ingredients. Regardless of the progress of the industry, it can't be replaced by some production process.
The quality of honey varies from year to year, and bees can never produce the same honey and honeydew. Weather conditions, grazing, treatment of bees, proximity to industry, roads, etc., greatly affect the quality of the obtained honey. Although minerals and heavy metals are minor constituents of honey, they play a vital role in determining its quality. The goal of the research is to assess the qualitative status of honey based on the content of contaminants, heavy metals from the area of the Tuzla Canton. The research was conducted on 30 (thirty) honey samples. The samples were collected in the period September/October 2022 and constitute the grazing of the specified year.
In the samples that were the subject of research, As and Cd did not exceed the limit of quantification (LOQ = 0.009 mg/kg). Current regulations does not define MRL's for these two metals. As for the quantified amount of lead (Pb), it was the same in 12 samples and in 11 samples there was an evident deviation from the MRL. The measured lead (Pb), values range from 0.06 to 5.34 mg/kg. The quality of bee products from the aspect of contamination with heavy metals can serve as bioindicator of environmental pollution, that is, as an indicator of the level of good beekeeping practices.

KEYWORDS: honey; honeydew; heavy metals; Tuzla canton

INTRODUCTION

Honey is primarily a concentrated sugar solution, composed mainly of glucose and fructose, together with other components such as organic acids, enzymes, vitamins, acetylcholine, flavonoids, minerals and trace elements [1]. The most common ingredients are carbohydrates, mainly fructose and glucose, and water, which together make up more than 99% of honey [2]. The biological activities of honey originate precisely from the compounds present in this natural food. In general, honey consists of approximately 200 different substances [3]. The chemical composition of honey is essentially related to factors such as the geographical region of origin, the flora of the region, the type of soil, the type of bee that produced it, the physiological state of the society, climatic conditions, processing conditions, handling, storage and storage time, the maturity of the honey [4]. The content of minerals and trace elements in honey can be used as an indicator of environmental pollution and an indirect indicator of the geographical origin of honey [5]. The determination of minerals, primarily heavy metals, in honey can be used to assess environmental contamination [6].

The chemical composition of honey from different botanical areas can vary. In this research, the focus is on the healthiness of honey that originates from the area of the Tuzla Canton. The aim of the research is to assess the quality of honey based on the content of contaminants with a focus on heavy metals (As, Cd, Pb) from the area of the Tuzla Canton..

The Tuzla canton is located in the northeastern part of Bosnia and Herzegovina, which in the south is characterized by a distinctly mountainous area (Konjuh, Javornik and Ozren), the valleys of the Spreča and Tinja rivers in the central part, while from the northwest to the southeast of the canton there are the massifs of Skipovac, Trebava and Majevica. [7]. Coal and rock salt are the two most important mineral resources of this region. The salt deposit is the only one of its kind in Bosnia and Herzegovina, while according to coal reserves, this area is the largest energy area of Bosnia and Herzegovina [8]. With over a billion tons of mineral reserves and a 100-year tradition of exploitation, the Tuzla Canton is the most important mining and industrial basin in Bosnia and Herzegovina. With 114,102 hectares of agricultural land, which is 49% of the total territory of the canton, agriculture is also a very important sector in Tuzla Canton [9].

The close source and product connection between plant-honey means that honey inherits different characteristics and shares biological properties with its corresponding botanical origin. [10]. For this reason, honey may contain undesirable compounds or remains of the original plant that was exposed to these substances, including those of anthropic origin. Among the residues that change the natural composition of honey are metals, which, depending on their concentration in food, can pose a risk to human health. An important aspect of honey quality is the presence of metals, which is directly related to the chemical composition of the soil in the areas where the bees feed. Therefore, the content of heavy metals in honey indicates contamination of the nearby soil, caused by volcanic and/or hydrothermal activities and weather conditions, among other factors that pose a risk to human health. [11, 12]. The usual route by which humans ingest and are exposed to metals is through the diet. Some heavy metals are essential elements for normal plant growth such as Co, Fe, Mn, Ni, Zn, and Cu and they play an important role in metabolism, but in higher concentrations the same metals become toxic. These increased levels can cause a decrease in the percentage of biomass in the plant and in many cases lead to the death of the plant. On the contrary, some heavy metals such as Pb, Cd, Cr and Hg are marked with high toxicity to plants [13].

Heavy metals are defined as metals whose density is greater than 5 g/cm^3. A total of 53 out of 90 natural elements are heavy metals [14]. In biology, the term "heavy" refers to a number of metals or metalloids that can be toxic to both plants and animals, even when their concentrations are very low. Soil contamination with heavy metals is different from water and air pollution, due to the fact that heavy metals remain longer in the soil than in water or air.[15].

The bee is active in the entire area around the hive although it is opportunistic in the sense that it prefers to gather pollen in nearby flower fields, the bee can move long distances, even up to ten kilometers in exceptional circumstances therefore the hive can maintain areas of seven square kilometers "under its control [16]. Honey can contain high levels of toxic elements, such as Hg, As, Cd and Pb as a result of increased amounts in plant nectar. Due to the large scale of mining and industrial activity, toxic metals are absorbed into the soil, atmosphere and water and consequently into plants. High concentrations of these metals were found in honey from areas with heavy industry, that is, near highways [17].

Cadmium is a non-essential, toxic element and is most often found in the soil in low concentrations, below 3 mg/kg. The availability of cadmium in the soil depends primarily on the type of soil, the form in which Cd is found in the soil, the content of organic matter, the pH of the soil and the cation exchange capacity. Soil contamination with cadmium can be caused by the use of mineral fertilizers, organic fertilizers and fertilizers derived from sewage sludge. Lead is a heavy metal found in exhaust gases from cars and factories. It is the main chemical pollutant of the surrounding environment. It appears in the form of ions Pb^{2+}, as lead tetraethyl, lead diethyl and as other alkyl derivatives of lead. The usual content of lead in agricultural soil is 2 to 100 mg/kg [15]. Among the most toxic elements present in nature is As. This element is widely distributed in nature, with the most important arsenic minerals being pyrite, realgar and orpiment. As concentrations found in soil are between 0.1 and 50 μg/g, with a mean value between 5 and 6 μg/g [18]. However, As levels can be much higher in soils polluted by human activity. Arsenic can accumulate in soil as a result of pesticide use, fertilizer application, and fossil fuel burning. Other As sources include industrial deposits and animal waste [19]. A significant part of As was produced by anthropogenic activity as a by-product of the formation of Cu, Pb, Co and Au. Gold minerals contain up to 11% As, while Pb and Cu minerals contain 2-3% As [20]. The toxicity of As varies depending on its chemical composition, whereby inorganic species are more toxic than organic species, and inorganic As is classified as a human carcinogen [21]. The Ordinance on Maximum Permitted Amounts for Certain Contaminants in Food ("Official Gazette of BiH", No. 68/14, 79/16, 84/18) prescribes the maximum permitted amounts of the following contaminants in food: nitrates, mycotoxins, metals, 3-monochloropropanediol (3-MPCD), polycyclic aromatic hydrocarbons, dioxins and dioxin-like polychlorinated biphenyls (PCBs), melamine and its analogues, accumulated radioactivity in the form of Cs134 and Cs137 and other metals: total arsenic (As), copper (Cu), iron (Fe) and nickel (Ni). The maximum permitted amounts for honey are defined as 0.1 mg/kg for lead, 2 mg/kg for copper and 20 mg/kg for iron.

MATERIALS AND METHODS

MATERIALS

The research was conducted on 30 samples of honey from the area of Tuzla Canton, i.e. from the areas of G. Tuzla, Gračanica, Gradačac, Kalesija, KladANJ, Lukavac, Srebrenik and Tuzla. The samples consisted of monofloral types of honey (linden, acacia), 13 of them, and polyfloral types of honey

(floral/meadow), 7 of them, and mixed honey, 10 of them in total.

The samples were collected in the period September/October 2022 and constitute the grazing of the specified year. The condition that the samples had to meet was that they were delivered from stationary pastures in a quantity of at least 500g and in glass packaging. The test was carried out in October 2022, and until the moment of the test, the samples were stored in controlled conditions, without temperature variations, without direct light, in a dry and airy place.

Table 1. Overview of the geographical and botanical origin of the samples

Sample's mark	Origin	Species
B1	Lukavac	Acacia
B2	Tuzla	
B3, B4, B5, B6, B7	Gračanica	
L1, L2	Tuzla	Linden
L3	Gradačac	
L4	Lukavac	
L5	Tuzla	
L6	Srebrenik	
P1	Kladanj	Polyfloral
P2, P3, P4, P5	Tuzla	
P6	Gornja Tuzla	
P7	Gradačac	
M1	Kalesija	Mixed (polyfloral and honeydew)
M2	Tuzla	
M3	Gračanica	
M4	Srebrenik	
MD1, MD3	Tuzla	Honeydew
MD2, MD4, MD5, MD6	Kladanj	

METHODS

The technique used to determine the content of heavy metals (Cd, As and Pb) is ICP - OES.

The apparatus on which the samples were measured is manufactured by PerkinElmer, and themodel is Optima 2100 DV. The ICP-OES instrument was invented by Stanley Greenfield (1964) and became an important analytical tool for the determination of about 75-90 elements from different samples.

Inductively coupled plasma is a stream of highly ionized argon that passes through the magnetic field of the coil. A high-frequency magnetic field ionizes argon, which is an inert gas, and plasma is formed. Plasma develops temperatures of 8000K – 10000K, which enables it to determine about 75 elements from the periodic table.

The technique used by ICP to measure samples is optical emission spectrometry (OES), i.e. the apparatus works on the principle of emission. When we introduce the sample into the plasma, which develops a high temperature, electrons are excited, which then go into an excited state. When returning to the basic state, light of a certain wavelength is emitted, which is measured on the detector. In order to transport the sample into the plasma, we must first disperse it, i.e. nebulize it, and we achieve this with nebulizers.

The gases used by ICP are argon, nitrogen and compressed air. Argon is used to form plasmaand clean the system of impurities when starting the device, while nitrogen is used to clean (purge) the optics. The compressed air removes the plasma tail and thus protects the optical parts from destruction.

RESULTS AND DISCUSSION

The origin of heavy metals in the soil can be anthropogenic or natural and can be related to different soil fractions, which determine the mobility and availability of these metals. Heavy metals such as Pb, Cd and toxic elements such as Cr, As could reflect the presence of pollutants due to environmental contamination or pharmacological (antiparasitic or acaricidal) treatment of honey or incorrect procedures during the processing and conservation stages of honey [22].

The honey bee (Apis mellifera L.) and its products are currently also used as bioindicators of environmental pollution. These insects fly around nectar plants that grow up to 4 km from the hive, but can travel up to 12 km, accumulating pollutants present in the air, soil and water [23]. For this reason, honey can serve as an indicator material to assess the contamination of the environment from which bees collected nectar to make honey.

Honey can also be contaminated during its processing by beekeepers, the equipment and tools used, and the process itself. Materials such as aluminum, stainless steel, and galvanized steel used in honey processing tools and equipment can leach some

metals (including Al, Cd, Co, Cr, Cu, Fe, Pb, Ni, and Zn) into honey [24, 25].

Table 2. Results of heavy metal content in honey samples

Species	Sample mark	Origin	*Concentration As (mg/kg)*	*Concentration Cd (mg/kg)*	*Concentration Pb (mg/kg)*
Acacia	*B1*	*Lukavac*	0,00	0,00	0,00
	B2	*Tuzla*	0,00	0,00	1,46
	B3	*Gračanica*	0,00	0,00	0,06
	B4	*Gračanica*	0,00	0,00	0,50
	B5	*Gračanica*	0,00	0,00	0,16
	B6	*Gračanica*	0,00	0,00	0,00
	B7	*Gračanica*	0,00	0,00	0,00
	N: 7	***Sr.vr.***	**0,0**	**0,0**	**0,3**
		SD	**±0,0**	**±0,0**	**±0,5**
Linden	*L1*	*Tuzla*	0,00	0,00	0,00
	L2	*Tuzla*	0,00	0,00	0,00
	L3	*Gradačac*	0,00	0,00	0,00
	L4	*Lukavac*	0,00	0,00	0,00
	L5	*Tuzla*	0,00	0,00	4,75
	L6	*Srebrenik*	0,00	0,00	5,04
	N: 6	***Sr.vr.***	**0,0**	**0,0**	**1,6**
		SD	**±0,0**	**±0,0**	**±2,5**
Polyfloral	*P1*	*Kladanj*	0,00	0,00	0,53
	P2	*Tuzla*	0,00	0,00	0,00
	P3	*Tuzla*	0,00	0,00	0,74
	P4	*Tuzla*	0,00	0,00	0,00
	P5	*Tuzla*	0,00	0,00	4,42
	P6	*G. Tuzla*	0,00	0,00	0,00
	P7	*Gradačac*	0,00	0,00	0,00
	N: 7	***Sr.vr.***	**0,0**	**0,0**	**0,8**
		SD	**±0,0**	**±0,0**	**±1,6**
Honeydew	*MD1*	*Tuzla*	0,00	0,00	0,00
	MD2	*Kladanj*	0,00	0,00	0,00
	MD3	*Tuzla*	0,00	0,00	0,00
	MD4	*Kladanj*	0,00	0,00	4,88
	MD5	*Kladanj*	0,00	0,00	0,00
	MD6	*Kladanj*	0,00	0,00	0,00
	N: 6	***Sr.vr.***	**0,0**	**0,0**	**0,8**
		SD	**±0,0**	**±0,0**	**±2,0**
Mixed	*M1*	*Kalesija*	0,00	0,00	2,43
	M2	*Tuzla*	0,00	0,00	0,00
	M3	*Gračanica*	0,00	0,00	0,00
	M4	*Srebrenik*	0,00	0,00	5,34
	N: 4	***Sr.vr.***	**0,0**	**0,0**	**1,9**
		SD	**±0,0**	**±0,0**	**±2,5**
Reference value			**ND**	**ND**	**max. 0,1**

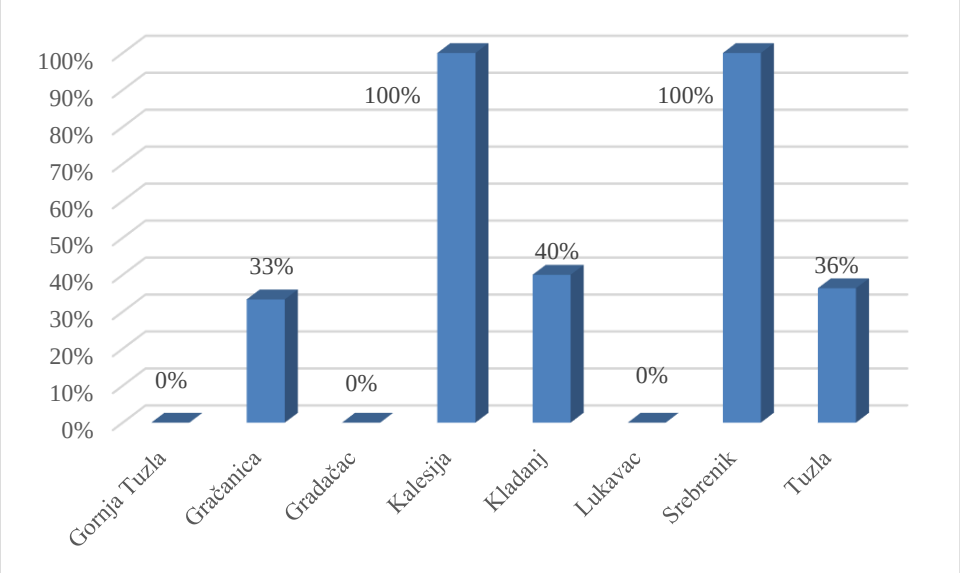
ND – not defined

In the samples that were the subject of research, As and Cd did not exceed the limit of quantification (LOQ), otherwise, for the method used, the limit of detection (LOD) was 0,003 mg/kg, and the limit of quantification was 0,009 mg/kg. Current regulations do not define MRLs for these two metals.

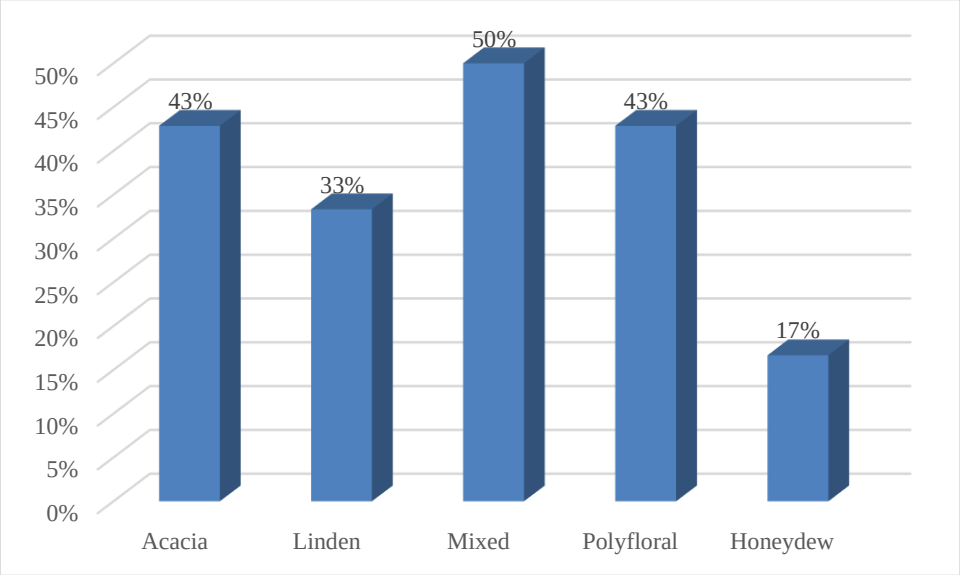
As for the quantified amount of lead, it was the same in 12 samples and in 11 samples there was an evident deviation from the MRL. It was quantified in all types of honey and from all areas except Gradačac and Lukavac and Gornja Tuzla. The measured lead values range from 0,06 to 5,34 mg/kg.

Headache, bad concentration and attention, irritability, memory loss can represent early symptoms of adverse effects exposure to Pb due to negative effect to the central nervous system [27]. Experimental studies have shown that Pb is potentially carcinogenic and is classified according to IARC as a probable carcinogen [21].

Based on the obtained results, two samples of acacia honey (B4 and B5) from the area of Gračanica contain an amount of lead above the permitted amount. Only one sample of acacia honey was analyzed from the Tuzla area, and the amount of lead above the prescribed value was detected in it. Two samples of linden honey (L5 and L6) contain an amount of lead above the permitted amount. Out of a total of six analyzed samples of medljikovac honey, one sample (MD4) from Kladnje contains an amount of lead above the prescribed value. Three (P1, P3 and P5) out of a total of seven samples of mixed honey contain more lead than the prescribed value. Samples P3 and P4 are from the area of Tuzla, while the third defective sample is from the area of Kladanj and is the only one from that region. In two (M1 and M4) out of a total of four samples of mixed honey, the lead content was quantified higher than the prescribed value, and these samples are from the area of Kalesija and Srebrenik.



Graph 1. Overview of % defective samples from the aspect of lead content by municipalities of Tuzla Canton



Graph 2. Overview of % defective samples from the aspect of lead content by botanical types from Tuzla Canton

Graph 1. shows that with regard to the municipalities of Gornja Tuzla, Gradačac and Lukavac, there are no samples with an increased concentration of lead in the tested samples. While all the samples that participated in the research from the areas of Srebrenik and Kalesija have an increased concentration of lead.

From graph 2., it is evident that the most samples of mixed honey have an increased concentration of lead, while the increased concentration of lead is the least in the honeydew samples.

Table 3. shows the results of other studies, which refer to the measured lead content in honey samples of different botanical species.

Table 3. Levels of heavy metals reported in honey samples from different countries [27]

Origin	France	Croatia	Italy	Israel	Malaysia	Romania	USA
Pb (µg/kg)	280.00 - 1080.00	4130.0 - 21590.0	100.00 - 1533.00	150.00 - 8220.00	nd - 1017.00	20.00 - 6000.00	<983.30 - 1534.80

nd – not defined

CONCLUSION

Lead (Pb), contained in the air and originating mainly from motor traffic, can contaminate the air, and then directly nectar and honeydew. In general, Pb is not translocated by plants. Proximity to industry, beekeeping and agricultural practices as well as other anthropogenic influences can be the source and consequence of elevated lead concentrations in food. The general conclusion is that the contamination according to the types of samples and according to the regions is not constant or consistent, but it certainly leads to the conclusion that a more detailed investigation is necessary. According to the variability of the results, it can be concluded that this contamination can be caused by the use of galvanized or welded metal fittings. Honey is an acidic product that can react with surfaces containing lead and cause migration of lead into the honey. Namely, the wire for fixing the clock bases can be galvanized or made of stainless steel (so-called chrome or stainless steel wire). Stainless steel wire can be used multiple times when replacing honeycombs and installing new clock bases, while galvanized wire must be changed every time when replacing honeycombs, i.e. installing new clock bases. The wire in the frames must not be rusted (corroded), so as not to cause chemical contamination of the bee products. The beekeeper's knife, equipment for inserting clock bases and queen grids should be made of stainless material (galvanized, plasticized, plastic or chrome-plated queen grid, etc.) or that they are not rusted, and that they are made of a material that allows them to be easily cleaned, washed and disinfected. All equipment for brewing, filtering, storage and packaging should also meet all minimum sanitary, technical and hygienic standards.

During the collection of samples, auditing of the implementation of good beekeeping practices was not carried out, since this was not an aspect of the research, but certainly the results of this research point to the need to record and investigate the impact of inadequate practices on the healthiness of the product.

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DYNAMICS OF SMOKE INGREDIENTS PENETRATION INTO THE INTERIOR OF CHICKEN MEAT PRODUCTS DURING BOILING/SMOKING AND DRYING

ORIGINAL SCIENTIFIC PAPER

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DOI: 10.51558/2232-7568.2023.17.1.11

RECEIVED 2023-10-27 **ACCEPTED** 2024-07-08

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ABSTRACT:

During processing in meat, numerous activities occur. Those activities can have a positive or negative impact on the properties of aw materials and finished products. The aim of this study was to determine the dynamics of smoke components penetration (total phenols and organic acids) during boiling/smoking and drying of chicken meat at different thermal regimes. The dynamics of total phenol and total organic acids penetration into meat samples was monitored during all stages of production (cooking/smoking, drying and storage). Eight experimental groups were analyzed. Samples in four experimental groups (EG I-IV) were subjected to boiling and smoking, and other four groups (EG V-VIII) to drying with different parameter values: boiling/smoking temperature 55-75°C, process duration 80-110 minutes, drying temperature 14-18°C, with air circulation 40-50 m^3/min and relative humidity 85-88% in appropriate conditions. Based on the obtained results, the statistically significant influence of different heat treatments and their technological parameters on the dynamics of phenol and organic acid penetration was established ($p<0.05$). Temperature stood out as an important technological parameter with the greatest impact. The results show that due to elevated temperatures during heat treatments there is an increase in the concentration of total phenols and organic acids. Furthermore, it was concluded that the investigated processing procedures of chicken meat had an impact on the dynamics of penetration of smoke components.

KEYWORDS: phenols, organic acids, boiling, smoking, drying

INTRODUCTION

Scientific research in the field of food safety has recently increasingly included research related to operations and processes of heat treatment, smoking and drying of meat in order to obtain a high quality safe product, with greater nutritional value and better sensory properties.

Heat treatment is a critical degree of meat processing. Three main factors are especially important: the temperature on the surface of the meat, the temperature in the center of the product and the method of heat transfer (contact, air or steam) [1], [2].

Smoking is a process in which the volatile components of smoke and steam from wood, which is not completely burned, penetrate the meat and act on certain ingredients of the meat. Smoking achieves specific sensory properties, primarily color and aroma [3], [4]. After smoking, the products have a pleasant, characteristic smell and taste of smoke and a light pink hue on the surface of the product [5], [6]. Sikorski and Kolakowski [8] and Roseiro et al. [8] state that 40-60% of meat products are smoked during production. The volatile components formed during incomplete burning of wood give a smoke preserving effect [4].

Smoke, which is created in the process of woodburning, contains several thousand different chemical compounds, some of which have a negative impact on human health. About 600 smoke constituents are known, which can be classified into different groups of organic compounds: aliphatic, ketone and aromatic acids, aldehydes, dialdehydes, aromatic aldehydes, alcohols, phenols, amines, aromatic hydrocarbons and others [9], [10].

Drying meat is a technological process, which aims to obtain quality products with a specific aroma and taste. Drying of meat leads to the formation of products with altered properties [9].

For the process of smoking/drying meat, the most important components of smoke are phenols, carbonyl compounds (aldehydes and ketones), organic acids and alcohols, which are formed at different temperatures. Of the phenols (formed at temperatures between 300°C and 550°C), cresols and creosote are important. Organic acids are formed at a temperature of 160°C to 250°C (the most important for changes in

smoked meat are: formic, acetic, propionic, butyl, benzoic) [8], [10]. During smoking process, the components that produce smoke are deposited on the surface of the product and diffuse to different depths in the products [11]. Typical sensory characteristics of smoked products were found to be associated with the presence of phenols and odoriferous substances resulting from various chemical and biochemical reactions in food [11], [12].

Several authors have examined the influence of heat treatment on the presence and content of phenols and organic acids in meat and meat products, but no research based on this kind of the meat and this kind of the processing [2], [4], [13], [14]. As mentioned, phenols are the main compounds associated with the desirable characteristics of the obtained products in various technological processes (dyeing, preservation and formation of flavors). The production of chicken meat is constantly increasing, so the production of smoked products from this type of meat is an opportunity to process surplus meat and better supply the population with specific products.

The aim of this work was to determine the dynamics of smoke penetration during boiling/smoking and drying of chicken meat with different heat regimes. Also, the impact of these heat treatments and processing time on the total phenols and organic acids concentrations.

MATERIALS AND METHODS

Chickens used during the experiment, were raised on the family farm in conditions of intensive breeding. Intensive breeding took place in two phases with appropriate feed mixtures (starter and finisher). The main ingredient in the feed mixtures that the animals were fed was predominantly corn. In addition to corn, toasted soybeans, sunflower and dehydrated alfalfa were added to the chicken mixes. The feeding of the chickens lasted 42 days. During the research, 48 hens were used. The breast meat (96 pieces divided in eight experimental groups) used for further research was shaped (6-7 cm wide and 12-13 cm long sample) and cooled for 24 hours at a temperature of about 1°C. After cooling (temperature <4°C was reached in the center of the piece), the pieces of meat were pickled (table salt 2.5%, nitrite salt 0.3%, pepper and garlic). The pickling process was carried out under controlled conditions: temperature 2-4°C, duration 10 days. After pickling, the samples were subjected to boiling (temperature between 55°C and 75°C, process time from 80 minutes to 110 minutes) and drying (temperature between 14°C and 18°C, with air circulation of 40 m^3/min up to 50 m^3/min and relative humidity from 85% to 88%) in appropriate conditions.

Experimental groups are divided based on changes in smoking/boiling parameters (EG I-IV) and drying (EG V-VIII). Twelve samples from each experimental group was treated by boiling (thermodynamic chamber for drying, smoking, baking and cooking by Atmnos Maurer Germany), and twelve (12) at low temperature were smoked and dried in a fermentation chamber (Universal thermodynamic chamber Doleschal, Austria) [4].

The values for the temperature and processing time for the experimental groups are as follows: EG I (temperature 70°C, time 90 minutes), EG II (temperature 65°C, time 100 minutes), EG III (temperature 60°C, time 100 minutes)), EG IV (temperature 55°C, time 107 minutes), EG V (temperature 17.5°C, time 300 minutes), EG VI (temperature 14.5°C, time 360 minutes), EG VII (temperature 14.5°C, time 240 minutes), EG VIII (temperature 14.5°C, time 300 minutes).

CHEMICAL ANALYSIS

Phenol penetration dynamics

Phenol penetration dynamics in chicken meat samples was monitored during the all phases of production (fresh samples, boiling/smoking and storage).The Folin Ciocalteu method as described in Naveena et al. [15] was used to determine the content of total phenols in chicken meat samples, 2008. In brief, 5 g of sample was homogenized with 25 mL of 70% acetone and kept overnight for extraction at 4°C. Aliquots of extracts were transferred in a test tubes and the volume was made to 0.5 mL with distilled water followed by the addition of 0.25 mL Folin–Ciocalteu reagent and 1.25 mL sodium carbonate solution (20%). The tubes were analyzed measuring the absorbance at 725 nm (WTW UV-Vis) after 40 min. The total phenolics were calculated as galic acid equivalent from the calibration curve using standard galic acid solution (0.1 mg/mL).

During the production of boiled products, the analysis of phenol content was performed on the first day (fresh meat), on the eleventh day (after first and second smoking) and on the twentieth day (storage). During the production of dried products, the determination of phenol content was performed on the first day (fresh meat), on the fifth and tenth (smoking) and on the twentieth day (storage). From each piece of meat, 15 pieces measuring 5 cm x 5 cm were cut (5 pieces from the surface, 5 from the line that forms the border between the zones of meat different in color shade and 5 from the center of the piece). Pieces from the same position were joined, chopped and a sample was taken for analysis. Phenol concentration was measured three times on each sample.

Organic acid penetration dynamics

The dynamics of organic acids penetration into chicken meat samples was monitored during all stages of production (fresh samples, boiling/smoking and storage) [16]. Meat samples were prepared in the same way as described in the previous section.

The content of total organic acids in chicken meat samples was determined by the method of neutralization with 0,1 M NaOH solution. Neutralization lasted until the indicator changed color after the addition of the first excess drop of alkali. During the production of boiled products, the analysis of organic acid content was performed on the first day (fresh meat), on the eleventh day (after I and II smoking) and on the twentieth day (storage). In dried products, the analysis of the organic acids concentration was performed on the first day (fresh meat), on the fifth and tenth day (smoking) and on the twentieth day (storage).

STATISTICAL ANALYSIS

The results of this study are presented as the mean values accompanied with their standard deviations. One factor analysis of variance (ANOVA) was performed using statistical software SPSS (VER.20). When the main impact was significant, averages were split by Tukey's test of the smallest significant deviations at 5% level. Level of significance ($p<0.05$) was used for comparison and discussion of the obtained results.

RESULTS AND DISCUSSION

Dynamics of phenol penetration in chicken meat samples

Tables 1 and 2 show the results of the phenol content in four experimental groups of chicken meat products during the technological production and during storage. The results of total phenols in samples of fresh chicken meat during the boiling process showed the highest concentration in EG I (131.62 mg/kg), and the lowest in EG IV (88.47 mg/kg). During the boiling process on the eleventh day (after I smoking) the highest phenol content was measured in EG I (212.64 mg/kg) and the lowest in EG IV (156.80 mg/kg). On the eleventh day (after II smoking), the highest phenol content was determined in EG I (173.14 mg/kg), and the lowest in the EG IV (142.53 mg/kg). On the twentieth day after storage of boiled chicken meat products, the highest mean value of the measured phenol content was in EG I (173.01 mg/kg) and the lowest in EG IV (144.00 mg/kg) (Table 1).

Table 1. Dynamics of phenol penetration in chicken meat samples during the technological processing (fresh meat, boiling/smoking) and after storage

Experimental group/Phenol concentration (g/kg)	Fresh meat	Boiling/Smoking		Storage
		First smoking	Second smoking	
	1. day	11. day	11. day	20. day
EG I	131.62±10.09	212.64±4.37	173.14±1.10	173.01±1.10
EG II	116.17±5.79	196.70±0.94	142.73±2.72	147.11±1.15
EG III	96.05±3.88	211.75±1.96	162.40±5.10	163.89±2.10
EG IV	88.47±1.37	156.80±1.45	142.53±3.15	144.00±7.08

* Data are presented as mean ± standard error from 6 samples (n = 6)

EG (I-IV)-Experimental group: EG I: 70°C, 90 min, 7.1 m^3/min, 74 -76 %; EG II: 65°C, 100 min, 9.7 m^3/min, 78-82 %; EG III: 60°C, 100 min, 9.7 m^3/min, 78-82 %; EG IV: 55°C, 107 min, 12.7 m^3/min, 82-86%

Using the t-test of paired samples with each other, the mean values of phenol content measured in different time periods (day 1, day 11, day 20) in all groups were compared. Statistically significant difference between the phenol content in EG I and EG II (t =5.90, Sig.=0.010), as well as the phenol content in EG IV (t=6.34, Sig.=0.008) was found, while the differences in phenol content in boiled chicken meat among other experimental groups were not statistically significant (Table 2).

Table 2. Results of phenol content comparison between experimental groups in chicken meat samples during the technological processing (fresh meat, boiling/smoking) and storage

Treatment	Test Value = 0					t	$\bar{X}_{dif}$	Sig.
	$\bar{X} \pm SD$	S.D.	Std.Error	95% CI differences Lower limit	95% CI differences Upper limit			
EGI - EGII	21.92	7.42	3.71	10.10	33.74	5.90	3	0.010
EGI - EGIII	14.08	14.96	7.48	-9.72	37.88	1.88	3	0.156
EGI - EGIV	39.65	12.50	6.25	19.75	59.55	6.34	3	0.008
EGII – EGIV	17.72	19.25	9.62	-12.90	48.36	1.84	3	0.163
EGIII - EGIV	25.57	20.42	10.21	-6.92	58.07	2.50	3	0.087

EG (I-IV)- experimental group, $\bar{X}$ – average value, S.D.-standard deviation CI (interval of trust), Sig. (p<0.05)

The results of determining the mean values of total phenol content in samples of fresh chicken meat used during drying showed that the highest phenol concentration in EG VII (113.34 mg/kg) and the lowest in EG VI (68.17 mg/kg). During the drying process on the fifth day of drying, the highest phenol content was measured in EG VII (194.25 mg/kg) and the lowest in EG V (128.44 mg/kg). After the tenth day of drying, the highest phenol content was determined in the samples from EG VII (202.15 mg/kg), and the lowest in the sample from EG V (138.11 mg/kg). On the twentieth day after storage of cooked chicken meat products, the highest mean value of the measured phenol content was in EG VII (201.11 mg/kg, and the lowest in EG V (141.17 mg/kg) (Table 3).

Table 3. Dynamics of phenol penetration in chicken meat samples during the technological processing (fresh meat, drying) and storage

Experimental group/Phenol concentration (mg/kg)	Fresh meat	Boiling/Drying		Storage
	1.day	5. day	10. day	20. day
EG V	70.69±3.22	128.44±1.48	138.11±9.00	141.17±1.10
EG VI	68.17±15.09	155.52±2.30	159.90±3.21	157.93±1.20
EG VII	113.34±10.40	194.25±1.07	202.15±5.10	201.11±3.45
EG VIII	108.96±2.53	174.56±5.77	186.23±4.11	187.16±1.10

*Data are presented as mean ± standard error from 6 samples (n = 6)

EG (V-VIII)-Experimental group: EG V: 17.5°C, 300 min, 40 m^3/min, 85-86 %; EG VI: 14.5°C, 360 min, 40 m^3/min, 85-86 %; EG VII: 14.5°C, 240 min, 40 m^3/min, 85-86 %; EG VIII: 14.5°C, 300 min, 40 m^3/min, 85-86 %

Using the t-test of the paired samples, the mean values of the phenol content measured in different time periods (day 1, day 5, day 10 and day 20) were compared. The obtained results showed a statistically significant difference in phenol content between dried chicken meat products during which different treatments were applied. The difference in phenol content in EG V and EG VI was not statistically significant (t=-2.44, Sig.=0.092), while the difference in phenol content in other treatments was statistically significant (Table 4).

Table 4. Results of phenol content comparison between experimental groups in chicken meat samples during the technological processing (fresh meat, drying/smoking) and storage

Treatments	Test Value = 0					t	$\bar{X}_{dif}$	Sig.
	$\bar{X} \pm SD$	S.D.	Std.Error	95% CI differences Lower limit	95% CI differences Upper limit			
EGV-EGVI	-15.77	12.90	6.45	-36.31	4.75	-2.44	3	0.092
EGV-EGVII	-58.11	10.59	5.29	-74.97	-41.25	-10.96	3	0.002
EGV-EGVIII	-44.62	4.34	2.17	-51.54	-37.70	-20.53	3	0.000
EGVI-EGVII	-42.33	2.69	1.34	-46.61	-38.04	-31.44	3	0.000
EGVI-EGVIII	-28.84	9.04	4.52	-43.23	-14.45	-6.38	3	0.008
EGVII-EGVIII	13.48	6.52	3.26	3.10	23.86	4.13	3	0.026

*EG (V-VIII) - experimental group , $\bar{X}$ – average value , S.D.-standard deviation CI (interval of trust), Sig. (p<0.05)

Dynamics of organic acid penetration in chicken meat samples

Tables 5 and 6 show the results of determining the organic acid content in four experimental groups of chicken meat products during the processing (in fresh samples, in boiled/smoked and smoked/dried samples) and finished products during storage. The results in the boiling process showed the highest value of organic acids in samples EG III (1.48%) and the lowest in EG IV (1.08%). During the boiling process on the eleventh day (after first smoking), the highest content of organic acids was measured in EG III (1.76%), and the lowest in EG I (1.56%). On the eleventh day (after second smoking) the highest content of organic acids was determined in EG II (1.62%), and the lowest in EG IV (1.38%). On the twentieth day after storage, the highest mean value of the measured organic acid content was in EG IV (1.00%) and the lowest in EG I (0.88%).

Table 5. Dynamics of organic acids penetration in chicken meat samples during the technological processing (fresh meat, boiling/smoking) and storage

Experimental group/ organic acid concentration (%)	Fresh meat	Boiling/Smoking		Storage
		First smoking	Second smoking	
	1.day	11. day	11. day	20. day
EG I	1.28±0.15	1.56±0.11	1.38±0.14	0.88±0.12
EG II	1.16±0.17	1.69±0.10	1.62±0.11	0.96±0.18
EG III	1.48±0.07	1.76±0.15	1.42±0.15	0.96±0.11
EG IV	1.08±0.13	1.71±0.22	1.40±0.10	1.00±0.15

*Data are presented as mean ± standard error from 6 samples (n = 6) EG (I-IV)-Experimental group: EG I: 70°C, 90 min, 7.1 m^3/min, 74 -76 %; EG II: 65°C, 100 min, 9.7 m^3/min, 78-82 %; EG III: 60°C, 100 min, 9.7 m^3/min, 78-82 %; EG IV: 55°C, 107 min, 12.7 m^3/min, 82-86%

Using the t-test of paired samples, the mean values of organic acid content measured in different time periods (day 1, day 11, day 20) in all experimental groups were compared. The obtained values were not statistically significant (Table 6).

Table 6. Results of organic acid comparison between experimental groups in chicken meat samples during the technological processing (fresh meat, boiling/smoking) and storage

Treatments	Test Value = 0							
	$\bar{X} \pm SD$	S.D.	Std.Error	95% CI differences		t	$\bar{X}_{dif}$	Sig.
				Lower limit	Upper limit			
EGI-EGII	-0.08	0.15	0.07	-0.32	0.15	-1.09	3	0.353
EGI-EGIII	-0.13	0.08	0.04	-0.26	0.00	-3.15	3	0.051
EGI-EGIV	-0.02	0.15	0.07	-0.27	0.22	-0.28	3	0.795
EGII-EGIII	-0.04	0.21	0.10	-0.38	0.29	-0.44	3	0.688
EGII-EGIV	0.06	0.11	0.05	-0.12	0.24	1.00	3	0.387
EGIII-EGIV	0.10	0.19	0.09	-0.20	0.42	1.08	3	0.358

*EG (I-IV) - experimental group , $\bar{X}$ – average value , S.D.-standard deviation CI (interval of trust), Sig. (p<0.05)

The results of measuring the mean values of organic acid content in samples of fresh chicken meat used during the drying process showed the highest content in EG V, and the lowest in EG VIII.

During the drying process on the fifth day, the highest content of organic acids was measured in EG V, and the lowest in EG VII. After the tenth day of drying, the highest content of organic acids was determined in the samples from EG VI, and the lowest in the sample from EG VII. On the twentieth day after storage, the highest mean value of the measured organic acid content was in EG VI and the lowest in EG VII (Table 7).

Table 7. Dynamics of organic acids penetration in chicken meat samples during the technological processing (fresh meat, drying) and storage

Experimental group/Organic acid concentration (%)	Fresh meat	Smoking/Drying		Storage
	1.day	5. day	10. day	20. day
EG V	1.48±0.21	1.51 ±0.05	1.76±0.15	1.77±0.17
EG VI	1.26±0.07	1.40±0.14	1.91±0.13	2.01±0.14
EG VII	1.35±0.16	1.36±0.17	1.44±0.05	1.44±0.43
EG VIII	1.03±0.16	1.51±0.10	1.82±0.19	1.98±0.19

*Data are presented as mean ± standard error from 6 samples (n = 6)EG (V-VIII)-Experimental group: EG V: 17.5°C, 300 min, 40 m^3/min, 85-86 %; EG VI: 14.5°C, 360 min, 40 m^3/min, 85-86 %; EG VII: 14.5°C, 240 min, 40 m^3/min, 85-86 %; EG VIII: 14.5°C, 300 min, 40 m^3/min, 85-86 %

Using the t-test of the paired samples, the mean values of the organic acid content measured in different time periods (day 1, day 5, day 10 and day 20) were compared. The obtained results showed that there is a statistically significant difference in organic acid content between treatments in dried chicken meat in EG V and EG VII (t=4.33, Sig.=0.027), while in other treatments there is no statistical significance (Table 8).

Table 8. Results of organic acid comparison between experimental groups in chicken meat samples during the technological processing (fresh meat, drying/smoking) and storage

Treatments	Test Value = 0					t	$\bar{X}_{dif}$	Sig.
				95% CI differences				
	$\bar{X} \pm SD$	S.D.	Std.Error	Lower limit	Upper limit			
EGV - EGVI	-0.01	0.21	0.10	-0.35	0.32	-0.13	3	0.898
EGV -EGVII	0.23	0.10	0.05	0.06	0.40	4.33	3	0.023
EGV - EGVIII	0.04	0.28	0.14	-0.40	0.49	0.31	3	0.772
EGVI - EGVII	0.24	0.32	0.16	-0.26	0.75	1.53	3	0.221
EGVI-EGVIII	0.06	0.14	0.07	-0.16	0.28	0.85	3	0.457
EGVII-EGVIII	-0.18	0.37	0.18	-0.78	0.40	-1.00	3	0.390

EG (V-VIII)-experimental group , $\bar{X}$ – average value , S.D.-standard deviation CI (interval of trust), Sig. (p<0.05)

The values of phenol content in fresh samples of chicken meat show an increase in the mean values of phenol content in all groups and a decrease on the eleventh day (second smoking) during boiling/smoking process. The value of phenol content in the tested samples during drying remained similar to the values obtained after the completion of the boiling/smoking process. The obtained values of phenol content in both processes of boiling/smoking and drying in the analyzed samples of final products increase during the process, the value of which after storage remained mostly similar. The obtained results are much higher in relation to the values (46.97%) obtained by Krvavica et al. [17], Nadia and Amal [14] (38, 87%), and Valø et al. [18]. The reason may be the formation of phenol and deposition on the surface due to elevated temperatures and longer boiling/smoking and drying.

Sérot et al. [19] found that the content of phenolic compounds in products increases with processing time and the applied temperature (55 minutes, 50°C), but the relative percentage of these compounds is constant for a given process and independent of process parameters, with which the results presented in this paper can be related.

The obtained results of the content of organic acids indicate an increase during the technological process. During storage obtained values remain unchanged, which is similar to the values presented in the paper Rekanović [2]. In fresh samples, the values of organic acids were lower compared to the values obtained after first smoking, where there was an increase in the mean values of organic acids. After the second smoke, there is a slight decrease in the average values of organic acids concentration, and the tendency to decrease continues during storage. During the smoking process on the fifth and tenth day, as well as during storage, there is a slight increase in organic acids in chicken meat products. The obtained results are similar to the values presented in their research by the authors: Rekanović, [2]; Rekanović et al. [20]; Nadia and Amal [14].

Furthermore, when the obtained values for the phenol and organic acids concentration in chicken

meat are taken into account, in the boiling process (after first smoking) there was an increase in the content of both phenol and organic acids. As the process went (after second smoking) there is a slight decrease in the share of these parameters whose value during and after storage remains approximately the same. The values obtained are similar to the values mentioned in the study of Rekanović [2].

During the entire process of drying and all stages of processing, the content of phenol and organic acids increases, and during storage no significant changes occur and the value remains approximately the same as the values obtained after the process. Both processes affect the obtained values, which means that the movement and penetration of smoke particles leads to a lesser extent into the final products. The reason may be prolonged exposure to smoke at elevated temperatures. Both processes affect the obtained values, which means that the movement and penetration of smoke particles lead to a lesser extent into the final products. The dynamics of penetration in all phases of processing has a slight tendency to increase the share of these components that are in line with the values of other research [14], [17], [18], [21], [22], [23], [24].

CONCLUSION

After the obtained and presented results, it can be concluded that the examined procedures of boiling/smoking and drying of meat affect (p<0.05) the penetration dynamics of smoke ingredients into the interior of meat sample by monitoring phenol concentration during processing and storage. Due to elevated temperatures, the concentration of total phenols increases. Furthermore, it can be said that the investigated procedures of boiling/smoking and drying meat affect (p<0.05) the dynamics of penetration of smoke ingredients into the interior of meat by monitoring the concentration of organic acids during processing and storage. Due to elevated temperatures, the concentration of organic acids increases. Temperature was singled out as an important technological parameter during the boiling/smoking and drying process with the greatest impact. The results show that due to elevated temperatures during heat treatments there is an increase in the concentration of total phenols and organic acids. During the storage process, the obtained values remain unchanged.

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BIOCIDAL PROPERTIES OF CuO NANOPARTICLES

ORIGINAL SCIENTIFIC PAPER

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DOI: 10.51558/2232-7568.2023.17.1.19

RECEIVED 2023-11-06 **ACCEPTED** 2024-07-08

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ABSTRACT:

Biocides are products used to prevent or control the spread of various harmful organisms such as bacteria or viruses. Silver and gold nanoparticles are mostly used as active substances of biocides used in the medical field. However, a more economically acceptable alternatives are different copper compounds, specifically copper(II) oxide. CuO nanoparticles were gained via sonication method from copper(II) acetate in a sodium hydroxide solution. Physical and chemical properties of gained CuO nanoparticles were investigated by X-ray diffraction analysis (XRD), energy dispersive X-ray spectroscopy (EDS), thermogravimetric analysis (TGA) and atomic force microscopy (AFM). Biocidal tests were performed on bacteria *Pseudomonas aeruginosa* and *Bacillus subtilis*, as well on fungi *Candida albicans* and *Aspergillus niger* using the disc diffusion method. The ultrasonic irradiation method was found to yield pure CuO nanoparticles smaller than 70 nm. Also, EDS measurement verified the stoichiometric distribution of copper and oxygen in the sample. Antimicrobial properties were proven excellent for both bacteria and fungi except for *Pseudomonas aeruginosa*, for which CuO nanoparticles seem to have low effect.

KEYWORDS: copper(II) oxide; sonication; antimicrobial properties

INTRODUCTION

In recent years, metallic and semiconductor nanoparticles are considered for use in biocides due to their versatile biocidal toxicity mechanism. [1], [2] Other properties which give added benefits to the use of these materials in nano-medicine field are surface area to volume ratio and physicochemical properties related to size and shape. Silver and gold nanoparticles are mostly used in this field, but copper(II) oxide is proposed as a more economical alternative. CuO nanoparticles possess a combination of high surface area, redox activity, and biocompatibility, rendering them promising candidates for applications in biomedicine, as well as water treatment and beyond. [3]–[6]

The sonochemical method, a powerful and versatile synthesis technique, uses the energy generated by high-frequency ultrasound waves to induce a range of chemical and physical transformations in liquids. The creation process of tiny, rapidly expanding and collapsing bubbles is known as acoustic cavitation and occurs because of the ultrasound waves. [7] These cavitation bubbles generate localized extreme conditions of temperature and pressure, leading to the formation of free radicals and the initiation of chemical reactions that might otherwise require a different synthesis approach. [8] The sonochemical method has found applications across diverse fields, including materials science, nanotechnology, and environmental remediation, due to its ability to facilitate processes like nanoparticle synthesis.

Antimicrobial resistance is a growing global concern due to the fast evolution of resistance mechanisms in microbes. Because of this, traditional biocidal agents, such as disinfectants, are showing diminishing efficiency. [9] In this context, CuO nanoparticles have emerged as a compelling solution due to their ability to induce potent antimicrobial effects through multiple mechanisms. These mechanisms include the generation of reactive oxygen species, disruption of cell membranes, and interference with vital cellular processes. [10]–[12] Such versatility in

their antimicrobial action makes CuO nanoparticles valuable tools for mitigating the spread of infections in healthcare settings, the food industry, and environmental remediation efforts.

This paper delves into the biocidal properties of CuO nanoparticles, exploring their preparation via the sonochemical method, inhibitory effects on various bacteria and fungi, and physical properties.

EXPERIMENTAL

SYNTHESIS

Copper(II) oxide nanoparticles were synthesized by a fast sonochemical method using a ultrasound tip CL-334 (Industrial Sonomechanics, USA) following the preparation method of Sonia et.al. [13]A typical synthesis involves the preparation of 0.02 mol solution of NaOH (p.a.) and 0.01 mol solution of copper(II) acetate (p.a.). The solutions are mixed separately for 30 min to obtain good homogenization and ensure complete dissolution of precursors. Then, the sodium hydroxide solution was added dropwise to the copper(II) acetate solution. The resultant mixture was then sonicated for 1 h at room temperature and a black colored precipitate was obtained. To acquire only CuO nanoparticles, the product was washed with water and ethanol in three cycles using sonification and centrifugation. The final step is the drying of the precipitate overnight at 60°C.

CHARACTERIZATION

The phase composition of the samples was verified by X-ray diffraction on Shimadzu XRD 6000 diffractometer operating in step scan mode with 0.02 °2θ step and 0.6 s retention time. For characterization, CuKα radiation (λ= 0.15405 nm) and a measurement range of 5 to 80 °2θ was used. The Scherrer equation (1):

$$d = \frac{k\lambda}{\beta cos\theta} \quad (1)$$

was implemented for the determination of the crystallite size of the prepared nanoparticles. Scanning electron microscopy enabled insight into sample morphology, which, paired with energy dispersive X-ray spectroscopy, provides the means to observe and determine surface elemental composition. The analysis was accomplished using Tescan Vega 3 scanning electron microscope coupled with Oxford INCA X-sight EDS detector operating at 10 kV. Atomic force microscopy was conducted in ambient conditions using CoreAFM microscope with a Tap300Al-G probe in a tapping mode. Nominal resonant frequency of 300 kHz, nominal spring constant of 40 Nm^{-1} and tip radius less than 10 nm proved best for the acquisition of quality images.

ASSESSMENT OF BIOCIDAL ACTIVITY

The biocidal effect was tested using the well diffusion method. The following microorganisms were used in this work: Gram-positive bacteria *Bacillus subtilis* 3020, Gram-negative bacteria *Pseudomonas aeruginosa* 3011 and the fungi *Aspergillus niger* 405 (mould) and *Candida albicans* 159 (yeast). All microorganisms are kept in the Microorganism Collection of the Faculty of Chemical Engineering and Technology, Zagreb, Croatia. The volume of Mueller-Hinton agar (MHA) poured into a Petri dish was 20 mL with a thickness of approximately 3 mm. 100 µL of a microbial culture suspension (0.5 McFarland) was applied to the surface of the MHA. A hole with a diameter of 6 mm was then punched aseptically with a sterile cork borer and a volume of 50 µg of the antimicrobial suspension was added to the well at various concentrations. The Petri dishes were incubated at 37 °C for 24 hours and the bacterial and fungal inhibition zones were assessed after 24 and 72 hours, respectively.

RESULTS AND DISCUSSION

The success of the sonochemical process for the synthesis of copper(II) oxide material was visible already 5 minutes after the commencement of the synthesis when the starting solution turned from bright blue to dark brown (almost black), indicating that the process of transformation from $Cu(CH_3COO)_2$ to CuO begins. The chemical reactions occurring in the sonochemical process are as follows: [13]

$$\begin{aligned} &Cu(CH_3COO)_2 + 2NaOH \\ &Cu(OH)_2 + 2CH_3COONa \rightarrow \\ &CuO + H_2O + 2CH_3COONa \end{aligned} \quad (2)$$

The diffractogram pattern shown on figure 1 is a match with ICDD card no. 89-2529 which confirms the formation of copper(II) oxide. All

maxima present in this figure are attributed to CuO, where the three highest diffraction maxima are for the Miller indices (111), (022) and (202). Since there are no other unaccounted diffraction maxima, it can be concluded that copper(II) oxide is the only crystalline phase in the sample. It is noteworthy that the maxima for the (111) plane at 35.44 °2θ and (002) plane at 38.58 °2θ show greater intensity than all other maxima in this sample. This usually occurs when a sample has a preferred orientation, i.e. layered structures or some type of elongation along one plane (nanofiber). Value for width at half height of the 38.58 °2θ peak was implemented in the Scherrer equation and 27.37 nm crystallite size was calculated. CuO maxima on figure 1 are narrow and of relatively high intensity, which is in concurrence with the calculated crystallite size.

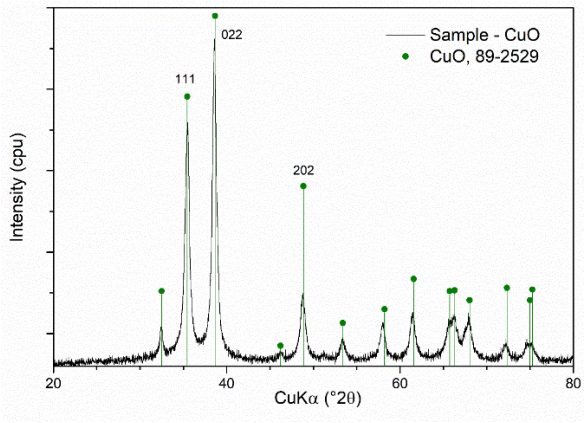

Figure 1. X-ray diffraction pattern for the sonochemically prepared sample

The easiness of the preparation of CuO nanoparticles via the sonochemical method is seen in the timing of the synthesis, which is only 1 h. As for the washing of the percipitate, cheap and easy to access chemicals are used and the longest part of the synthesis is the 24 h drying. Figure 2 shows the EDS mapping of a small part of the sample. Figure 2a refers to the sample and shows that areas of agglomeration of CuO nanoparticles appear. It can also be seen that these agglomerates are not compact but, on further investigation, look fluffy. EDS is a good tool for determination of the elemental composition of the sample surface.

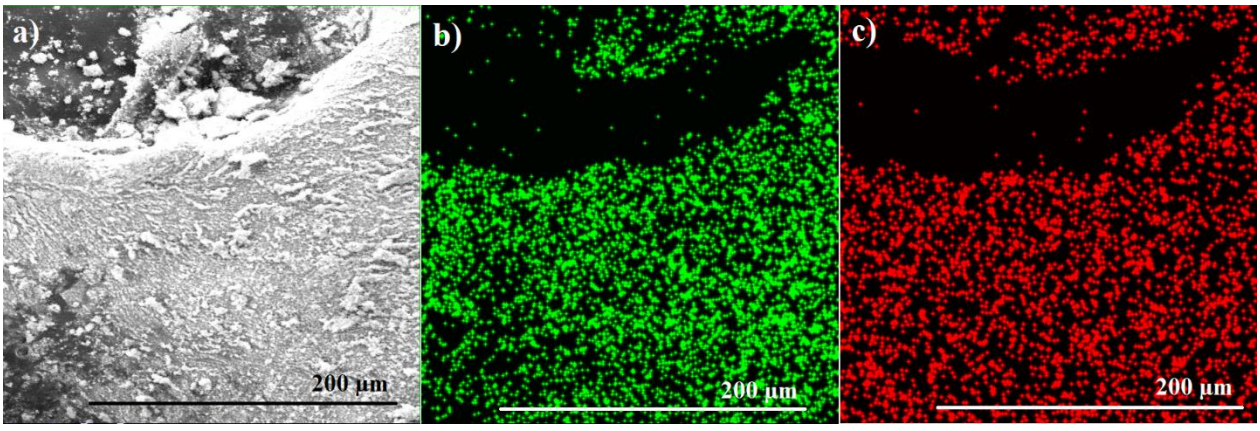

Figure 2. a) SEM micrograph, EDS mapping and distribution of: b) copper c) oxygen

In the images obtained by EDS mapping of the sample CuO, it can be discerned that the elements copper (2b) and oxygen (2c) are present in the sample. Trace amounts of gold, palladium and carbon are also present due to sputter coating of the sample with Au and Pd, and its deposition on carbon tape.

Table 1. Elemental composition of copper (II) oxide sample gained by EDS mapping

Element	Cu	O
Atomic number	29	8
Mass percentage (%)	47.40	25.60
Atomic percentage (%)	42.26	57.74
Relative error (%)	3.34	3.57

It can be seen that the areas of occurrence of copper and oxygen coincide, which is a confirmation of the chemical composition of the obtained crystalline phase. In addition, the results of the analysis are given in a tabular view (table 1). When viewing table 1, an interesting phenomenon is noticed. The atomic ratio of copper to oxygen should be 1:1 because of the chemical formula, CuO, however, there is a slight increase in the percentage of oxygen compared to copper. This is probably due to the nature of nanoparticles which, thanks to their great specific surface area, are susceptible to contamination in the form of adsorption of water and carbon dioxide from ambient air. AFM imaging was used to get insight into particle size of the prepared CuO nanoparticles.

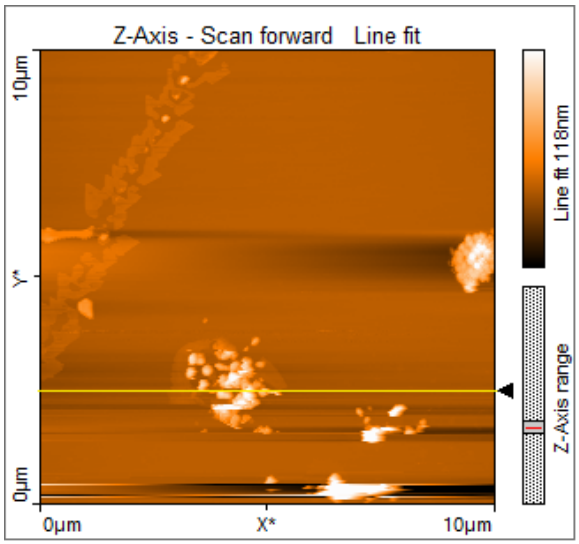

Figure 3. AFM micrograph of prepared CuO sample

Figure 3 represents the morphology of the sample where a very small portion of the powder sample was mixed with ethanol, deposited on a mica surface and left to air dry. The feature in the upper left corner is a crack created during the separation of mica layers, while the particulate parts in the image (in the middle and on the right) represent the CuO nanoparticles. Due to great surface energy, nanoparticles are prone to agglomeration and therefore, in this case, AFM turned out to be unsuitable for particle size estimation. On the other hand, the z-axis profile, due to a technique used for sample deposition, could provide insight into particle size. A z-axis height profile is given on figure 4 representing the height profile of the sample taken across the yellow line in figure 3.

The average height of CuO nanoparticles calculated from the z-profile is 44.5 nm.

Along with the particle height, which is an indication of particle size, assuming spherical particles, this figure also gives insight into particle distribution on the surface. Taking the x-axis dimension into consideration on figures 3 and 4 the claim on particle agglomeration gains credibility.

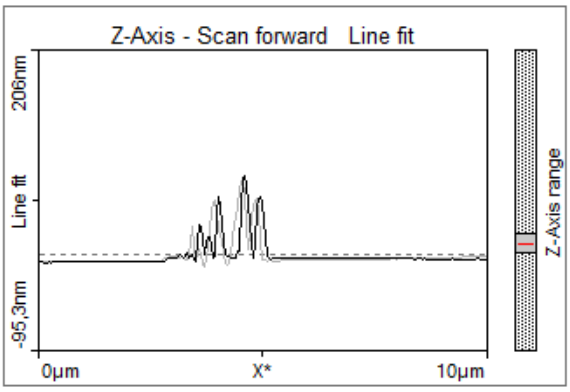

Figure 4. Height profile of particles across the yellow line in figure 3

CuO nanoparticles are known to have good biocidal properties, they mostly manifest great inhibition of Gram-positive bacteria, such as *Bacillus subtilis* or different fungi. Inhibition zones of prepared nanoparticles were tested on *Pseudomonas aeruginosa* 3011 and *Bacillus subtilis* 3020, as well as on *Candida albicans* 159 and *Aspergillus niger* 405. For the tests, different concentrations of CuO were dispersed in water. Table 2 shows the results for concentrations up to 100 mg L^{-1} for all tested microbes. As can be observed in Table 2, *Bacillus subtilis* 3020 and *Candida albicans* 159 show the greatest sensitivity to copper(II) oxide nanoparticles, as seen from the widest inhibition zones during disc diffusion tests. Given that microbial pores are greater than nanoparticle size, it is assumed that nanoparticles are able to infiltrate the cell membrane of this microbe, cause damage and disrupt cross-linking between nucleic acid strands inside the cell. [14] On the other hand, *Pseudomonas aeruginosa* 3020 seems to be

completely insensitive to copper(II) oxide nanoparticles, while *Aspergillus niger* 405 is sensitive only to the greatest concentration of nanoparticles. It is noteworthy that even the greatest concentration has no effect on the Gram-negative *Pseudomonas aeruginosa* 3011. That was a surprise given the work of Khashan et.al [15], whose findings indicated that CuO nanoparticles with average particle size of about 15 to 20 nm have greater inhibition effect on Gram-negative (*E. coli*, *P. aeruginosa* and *P. vulgaris*) than on Gram-positive bacteria. However, the concentration used in the work of Khashan et.al [15] was much greater than that used in this work, so another set of tests was conducted with greater concentrations. Indeed, the greatest concentration used, 1000 mgL^{-1} proved to be effective for the Gram-negative bacteria.

Table 2. Designations for different concentration of CuO nanoparticles with belonging inhibition zones for *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans* and *Aspergillus niger*

Designation	Sample concentration (mgL^{-1})	*Pseudomonas aeruginosa* (mm)	*Bacillus subtilis* (mm)	*Candida albicans* (mm)	*Aspergillus niger* (mm)
A	0.1	-	8	10	-
B	1	-	11	11	-
C	10	-	14	13	-
D	100	-	16	15	15

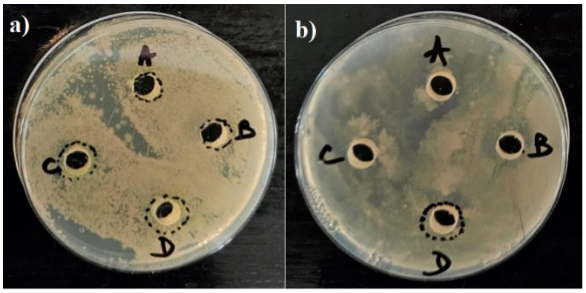

Figure 5. Disc diffusion tests for a) *Candida albicans* and b) *Aspergillus niger* for concentrations given in table 2

Table 3. Designations for different concentration of CuO nanoparticles with belonging inhibition zones for different bacteria strains

Designation	Sample concentration (mgL^{-1})	*P. aeruginosa* (mm)	*B. subtilis* (mm)
A1	250	-	18
B1	500	-	22
C1	750	-	25
D1	1000	20	25

At this concentration, the inhibition zone is greater than inhibition zones of all lesser concentrations for all the microbes tested. This behavior is peculiar because there is a minimum concentration of an antibacterial material below which the material has no effect on that specific microbe. *Bacillus subtilis* 3020 shows an improvement of the inhibition zone with higher concentration, but with stagnation above 750 mg L^{-1}, which can be considered the maximum inhibition zone. The difference between the antibacterial effect on Gram-positive and Gram-negative bacteria could result from a lower interaction potential for Cu^{2+} ions bonding to Gram-negative bacteria having an outer membrane containing lipopolysaccharide, which Gram-positive bacteria lacks. [16]

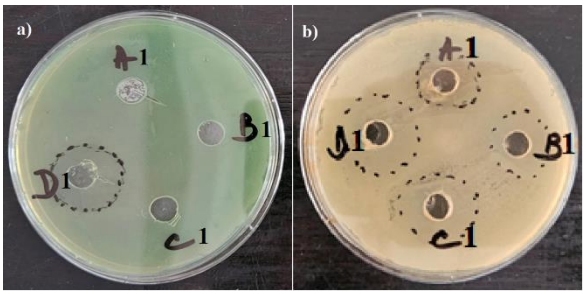

Figure 6. Disc diffusion tests for a) *Pseudomonas aeruginosa* and b) *Bacillus subtilis* for concentrations given in table 3

CONCLUSION

The sonochemical method of synthesis proved to be an effective and fast method of preparing copper(II) oxide nanoparticles. Nanoparticles were proven to be less than 40 nm in size. As for the antimicrobial tests, the prepared nanoparticles showed very good results for *Candida albicans* 159 and *Bacillus subtilis* 3020. CuO nanoparticles have less effect on mold and show a marginal effect on Gram-negative *Pseudomonas aeruginosa* 3011 only at high concentrations.This could be because Cu^{2+} ions bond to the bacterial cell, disrupting the biochemical processes inside the cell.

The poor antibacterial effect on Gram-negative bacteria could be the result of a lower Cu^{2+}ions interaction potential with Gram-negative bacteria due to its outer membrane containing lipopolysaccharide.

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EXTRACTION OF OILS FROM FRUIT KERNELS WITH CONVENTIONAL AND INNOVATIVE METHODS: A REVIEW

SCIENTIFIC REVIEW ARTICLE

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DOI: 10.51558/2232-7568.2023.17.1.25

RECEIVED 2023-11-21 **ACCEPTED** 2024-05-10

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ABSTRACT:
Over recent years, the food industry has striven to reduce waste, mostly because of rising awareness of the detrimental environmental impacts of food waste. While the edible oils market is enlarging constantly, there is increasing interest in producing plant-based oils because of the presence of various bioactive components like mono and poly unsaturated fatty acids. In recent publications it has been shown that various fruit kernels represent source of these components while the conversion of bio-waste into valuable compounds is of outmost importance for ensuring sustainability of the environment. This review investigates the different methods used for extraction of oils from sour cherry, peach, apricot and olive kernels and comparison between these methods in terms of extraction yield, fatty acids profile, tocopherols yield and antioxidant activity. An overview of chemical composition, bioactivity and on the application of these oils in end markets such as cosmetics, pharmaceuticals and nutraceuticals is presented. Scientific databases such as PubMed, Scopus, Google Scholar, Research Gate, ClinicalTrials have been used to assemble the data for this review.

KEYWORDS: extraction, oil, sour cherry, apricot, peach, olive

INTRODUCTION

The expansion of the world population has caused higher demand for food, resulting in the constantly expanding production of various fruits, vegetables and cereals. Consequently, with increased food processing, there has been a significant growth in agricultural waste leading to adverse environmental effects and economic losses. Up to 30% of all crops are discarded amounting to hundreds of thousands of tons annually of discarded fruit across the sector.
In the literature, various fruit wastes, including seeds, peels, pomace, stems, leaves, and stones were evaluated according to their chemical compositions, and remarkable amounts of bioactive components were identified [1]-[5]. Besides fruit wastes, the kernels of some tree crops apricot, almond, walnut, hazelnut, peach can also be utilized for oil extraction purposes for edible and non-edible uses. The oils of tree fruits and kernels are also becoming popular very fast for various foods, pharmaceutical and cosmetic industries [6].

The extraction of bioactive components from various fruit wastes ensuring high extraction efficiency without further utilization of hazardous chemicals is challenging in order to improve the sustainability of the food system [7].

Conventional extraction methods are generally based on an organic solvent, often confronted as liquid−liquid or solid−liquid extraction methods. On the one hand, in addition to organic solvent usage, another disadvantage of these techniques is the incorporation of an evaporation step, which cannot be ignored due to a high possibility of thermal destruction of bioactive components [8]. On the other hand, novel "green" methods recently emerged in the literature, which can be listed as microwave-assisted extraction (MAE), pulsed electric field (PEF), ultrasound-assisted extraction (UAE), supercritical fluid extraction (SC-CO_2), enzyme-assisted extraction (EAE), and pressurized liquid extraction (PLE) [9]. A proper extraction method should be chosen with outmost care to enable careful extraction with no chemical alteration.

In this regard, this review emphasizes the methods used for oil extraction from kernels of sour cherry, apricot, peach and olives, chemical composition and antioxidative activity. Cosmetic, nutraceutical and pharmaceutical applications of value-added products is also given.

METHOD

Searches of databases including PubMed, Scopus, Google Scholar, Research Gate, ClinicalTrials for scientific research papers were conducted. The keywords and terms used included extraction of oil and combinations of sour cherry, peach, apricot, olive kernel. As appropriate papers were identified, further search terms used were specific to industry sectors and applications. The papers selected were restricted to those published in English language. No geographical restrictions were applied. Some industry sectors and concepts were explored further using publicly accessible websites. To identify appropriate clinical trials, searches were conducted of the database ClinicalTrials.gov (U.S. National Library of Medicine). The search terms used were oil and sour cherry, peach, apricot and olive.

EXTRACTION OF OIL FROM SOUR CHERRY KERNEL

Sour cherries, species of *Prunus* in the subgenus *Cerasus*, are grown throughout the world at amounts about 1.2 million tons/year [10]. Sour cherries are mostly consumed as processed products, such as canned and frozen sour cherry, or sour cherry juice. During processing of sour cherries, high amounts of kernels arise as a waste material that could be used as a dietary fiber, protein, and fat source [11].

In the available literature different methods for extraction of oils from sour cherry kernel have been used starting from the conventional like cold press, Soxhlet extraction and innovative like superfluid extraction [12]-[24]. The dominant method used is Soxhlet with nonpolar solvents like n-hexane and petroleum ether.

Table 1. Extraction of sour cherry kernel oil

Extraction yield (%)	Method	Fatty acid composition (%)							Location	Reference
		C18:1	C18:2	C18:3	C16:1	C16:0	C18:0	C20:0		
ND	SE	43.9	44.8	0.48	0.44	7.8	2.4	0.73	Iran	[12]
ND	SE	52.9	35.0	ND	0.3	7.6	2.3	1.4	Canada	[13]
ND	SE	42.9	38.2	ND	ND	11	6.4	0.9	Romania	[14]
ND	SE	46.80±0.16	40.58 ±0.13	5.06 ±0.14	ND	6.23 ±0.15	1.33 ±0.13	ND	Turkey	[15]
ND	SC-CO_2	44.99±1.38	41.81 ±0.13	4.63 ±1.09	ND	7.24 ±0.16	1.33 ±0.12	ND		
ND	CP	35.45	42.34	0.13	0.50	6.54	2.03	0.87	Iran	[16]
ND	CP	37.89	42.42	0.11	0.29	4.92	1.60	0.64	Turkey	[17]
30.9	SE	47.62	33.47	0.12	0.63	8.18	2.46	0.90	USA	[18]
ND	SE	45.03±0.06	40.61 ±0.07	3.87 ±0.06	ND	5.93 ±0.04	3.3 ±0.05	1.26±0.37	Iran	[19]
32-36	SE	50-53	35-38	ND	ND	3-4	ND	ND	Hungary	[20]
55.95± 2.70	CP	35.28 ±2.16	40.19 ±1.97	ND	1.32 ±0.36	19.5 ±4.17	1.31 ±0.11	ND	Turkey	[21]
17.5-31.8	SE	25.3-45.3	35.5-46.1	0.09-0.48	0.16-0.33	5.1-7.4	2.2-3.4	1.0-1.4	Latvia	[22]
4.00	CP	41.92	46.82	0.33	0.38	6.35	2.27	1.10	Serbia	[23]
5.15	SE	41.46	47.00	0.33	0.37	6.62	2.21	1.12		
13.02	SC-CO_2	40.80	47.37	0.32	0.37	6.91	2.29	1.09		
ND	SE	46.9	41.7	/	/	9.4	2.0	ND	Bulgaria	[24]

The fatty acid composition is analyzed (Table 1) and the presence of various bioactive components (Table 2). Sour cherry kernel oil is rich in monounsaturated oleic acid and polyunsaturated linoleic acid (higher than 80%) while the dominant saturated fatty acids are palmitic and stearic (less than 15%). Other fatty acids like linolenic, palmitoleic, arachidic were found in very low concentrations.

Yilmaz and Gokmen [15] used two methods of extraction, namely Soxhlet and SC-CO_2 in which it was shown that the extraction method did not have a significant effect on the fatty acid profile. The study of Gornaś et al. [21] showed that the quantity of fatty

acids is affected by the cultivar of sour cherry. The difference in the composition of fatty acids in the oils extracted is due to different geographic locations and conditions in which the plants are grown.

Only in a few publications [16], [17], [22] the presence of α-eleostearic acid is reported. In sour cherry kernel oil of the cultivar Tamaris the presence of this fatty acid was 15.76% [22]. The compound is found to induce programmed cell death of fat cell [25] and of HL60leukemia cells in vitro at concentration of 20 μM [26].

BIOACTIVE COMPOUNDS IN SOUR CHERRY KERNEL OIL

Vitamin E is a group of eight compounds: α-, β-, γ-, δ-tocopherols and α-, β-, γ-, δ-tocotrienols, which are lipid-soluble [27]. All vitamin E isoforms, have antiproliferative, pro-apoptotic, anti-angiogenic, and anti-inflammatory effects [28].

Sour cherry kernel oil is rich in tocopherols and tocotrienols as it is shown in above cited articles, among them the predominant is γ-tocopherol [15]-[18], [21], [22]. Two tocotrienols α (0.5-2.2 mg/kg oil) and γ (0.1-0.4 mg/kg oil) were detected in sour cherry kernel oil in a study conducted by Gornaś et al. [22].

Phytosterols compounds are biologically active molecules with multiple health application such as reducing total and low-density lipoprotein (LDL) cholesterol levels [29]; have antioxidant, antiulcer, immunomodulatory, antibacterial, and antifungal effects [30].

Kazampour-Samak et al. [16] found that the most abundant sterol compounds were β-sitosterol (83.55%), Δ5-avenasterol (6.8%), sitostanol (4.8%), campesterol (3.5%), and stigmasterol (0.53%), respectively.

The sterol composition of sour cherry kernel oil consisted of 88.69% of β-sitosterol in total of thirteen sterols identified in the study of Atik et al. [17].

Nine sterols (campesterol, D-sitosterol, D5-avenasterol, 24-methylene-cycloartanol, cholesterol, gramisterol, D7-stigmasterol, D7-avenasterol, and citrostadienol) were quantified in a study of kernel oils of six sour cherry cultivars [22].

Table 2. Bioactive compounds present in sorry cherry kernel oil

Method	Tocopherols (mg/kg oil)				Sterols (%)			Carotenoids (mg/100 g oil)	Reference
	α	β	γ	δ	β-sitosterol	Campesterol	D5-avenasterol		
SE	71.6		298.7	96.9		ND		9.20	[15]
SC-CO_2	96.62		330.84	41.99		ND		5.93	
CP	325.00±3.29	ND	470.00±5.22	37.50 ±2.22	83.55±5.28	3.50 ±0.11	6.80 ±0.18	ND	[16]
CP	102.58	4.56	70.62	46.67	88.69	2.76	2.61	ND	[17]
SE	61.0	ND	400.0	64.2	44.5	2.55	ND	ND	[18]
CP	39.07± 0.23	ND	701.42±2.73	76.15 ±0.26	41	1.85	2.16	ND	[21]
SE	9.2-38.5	0.5-2.5	89.1-133.3	9.5-18.2	24.1-85.2	0.76-4.16	0.15-7.82	0.51-1.75	[22]
CP	6.39±0.08	1.07±0.25	25.22±0.05	5.41±0.18	ND	ND	ND	ND	[23]
SE	4.71±0.28	0.93±0.09	26.06±1.56	5.68±0.18	ND	ND	ND	ND	
SC-CO_2	6.25±0.34	1.00±0.06	23.85±1.46	5.13±0.25	ND	ND	ND	ND	

Carotenoids consumption in human diet reduces the risk of a variety of chronic illnesses, including cardiovascular diseases and neurological disorders, type 2 diabetes, and different types of cancer [31].

The findings of Yılmaz and Gokmen [15] proved that extraction method had an impact on β-carotene content in sour cherry kernel oil. Hexane extracted oil had significantly higher levels of β-carotene compared to oil extracted with SC-CO_2.

In sour cherry kernel oils from Latvia [22] only minor content of carotenoids were determined, with an average value of 0.94 mg/100 g oil and significant amounts of squalene (65.8–102.8 mg/100 g oil).

Phenols are bioactive compounds capable of scavenging free radicals and antioxidant activity. These compounds are abundantly found in plants and, as secondary metabolites, play an important role against oxidative stress [32].

Total phenolics were determined in the study of Yilmaz and Gokmen [15] which were in the range from 6.60 mg GAE/L to 27.87 mg GAE/L. The oil extracted with SC-CO_2 had higher content of total phenolic compounds.

The total phenolic content in extracted oil of sour cherry kernel was 33.44 mg GA/g dry matter [16].

Ten phenolic compounds were identified [17], phenolics acids levels were higher than other phenolic compounds. Benzoic acid (79.7 mg/kg), vanillin (5.62 mg/kg), p-coumaric acid (2.80 mg/kg), apigenin (1.82 mg/kg) were most abundant phenols in the SCKO.

EXTRACTION OF OIL FROM APRICOT KERNEL

Apricot (*Prunus armeniaca*), a member of the *Rosaceae* family, has been widely cultivated in Mediterranean countries as well as in Russia, Pakistan, the United States, and Iran [33]. The apricot kernel is a byproduct of apricot fruit and can be eaten as an appetizer (either raw or roasted) [34]. Nevertheless, the kernel is especially important for the oleo-chemical industry due to its valuable oil.

Table 3. Extraction of apricot kernel oil

Extraction yield (%)	Method	Fatty acid composition (%)							Location	Ref.
		C18:1	C18:2	C18:3	C16:1	C16:0	C18:0	C20:0		
ND	SE	64.4	26.9	0.1	0.8	6.7	1.1	ND	Bulgaria	[24]
39.8	SE				ND				India	[35]
Hexane: 47.8 Ether: 39.2 Acetone: 46.0 Ethanol: 30.3 Chlo:Met 54.6	SE	63.3-72.8	21.3-29.0	ND	0.5-0.8	4.0-5.7	1.2-1.5	ND	Turkey	[36]
32.44	CP-EAE				ND				India	[37]
35.6±0.97	CP	70.4 ±0.49	21.7 ±0.53	ND	0.6 ±0.03	6.1 ±0.13	1.2 ±0.08	ND	Turkey	[38]
45.9±0.34	SE	72.1 ±0.21	19.9 ±0.65	ND	0.5 ±0.06	6.4 ±0.04	1.1 ±0.07	ND		
36.78	CP	62.73	29.18	ND	ND	ND	ND	ND	Croatia	[39]
48.76	SC-CO_2	57.33	33.81	ND	ND	ND	ND	ND		
ND	SE	69.79 - 71.43	22.10-22.71	0.74-1.08	0.65-0.75	2.93-3.42	1.06-1.69	ND	Poland	[40]
32.9	CP	67.81	26.33	0.10	0.78	3.81	1.00	0.08	China	[41]
52	SE	67.96	26.21	0.13	0.69	3.83	0.99	0.07		
44	UAE	68.13	25.94	0.10	0.63	3.96	1.01	0.12		
52.7-54.4	SE	83.3	45.6	2.0	1.50	19	3.50	ND	Turkey	[42]
ND	SE	62.34 - 80.97	13.33-30.33	0.73-1.03	0.32-0.71	3.35-5.93	1.10-1.68	ND	Pakistan	[43]
ND	SE	58-65.7	29-33	0.5-1.0	1-2	4.6-6.0	0.5-1.20	0.2	USA	[44]
ND	SE	53.06 - 70.90	21.43-35.67	ND	ND	4.56-6.03	ND	ND	Turkey	[45]
ND	CP	70.90 ±0.00	20.93±0.00	0.74±0.00	ND	4.25±0.00	1.36±0.00	/	Macedonia	[46]

The apricot kernel oil contains more than 85% unsaturated fatty acids and less than 5% saturated fatty acids (table 3). Oleic acid is the main unsaturated acid followed by linoleic acid, while palmitoleic and linolenic only in few papers are reported with amounts less than 1%. The main saturated fatty acids are palmitic and stearic. In the published papers conventional methods like cold press and Soxhlet are mostly used for the extraction of oils from peach kernels with few reports of SC-CO_2 and ultrasonic [24], [35]-[46].

Iuata [38] showed that in comparison of two methods higher extraction yield was obtained with Soxhlet extraction (45.9%) simile to cold press (35.6%), but there was insignificant difference for fatty acid composition using two different extraction techniques.

In the study of Pavlović et al. [39] a comparison of the conventional cold press and the innovative SC-

CO_2 was performed. Higher extraction yield was obtained with SC-CO_2 (48.76%) to the cold press (36.78%) while not significant changes in the total content of unsaturated acids was observed.

Wang et al. [41] analyzed the three methods cold press, Soxhlet and ultrasonic assisted extraction. Among these higher efficiency of the extraction (52%) was obtained with Soxhlet using n-hexane as a solvent. The fatty acid profile was very similar in all the obtained oils.

Research group of Azcan and Demirel [36], Anwar et el. [43], Ozcan et al. [45] showed that the fatty acid composition of apricot kernel oil varies widely among different plant species.

BIOACTIVE COMPOUNDS IN APRICOT KERNEL OIL

Total tocopherols were determined in apricot kernel oil extracted with CP and SE methods. γ-tocopherol was determined as the main and the highest tocopherol isomer and α- tocopherol was determined as the secondary tocopherol isomer in both oils. δ- and β-tocopherol were determined in lower amount than the other isomers. The amount of γ- and α- tocopherol in CP-AKO was nearly 1.5-fold higher than that of SE-AKO. β- and δ- tocopherol in SE-AKO was higher than that of CP-ASO [38].

Pavlović et al. [39] obtained higher total content of tocopherols by CP, although α-tocopherol was extracted by SC-CO_2, while this was not achieved by the application of the cold press technique.

The results of the research presented [40] indicate that the apricot cultivar significantly influenced the content of tocopherols. Among the five tested apricot cultivars, the most valuable oil in terms of content of tocopherols was the oil obtained from the kernels of the 'Somo' cultivar.

In the study of Anwar et el. [43] tocopherol contents for apricot kernel oils exhibited a significant variation among the varieties analyzed. The highest concentration of α-tocopherol was exhibited for the apricot kernel oil of the variety Charmagzi (40.4 mg/kg) while the lowest was found for the variety Halmas (14.8 mg/kg). The contents of δ-tocopherol were found to be higher (60.2 mg/kg) in the variety Nari, whereas the lowest (28.5 mg/kg) was found for the variety Halmas, among others.

Rudzinska et al. [47] demonstrated in their study that AKO is a good source of diverse phytosterols (215-973.6 mg/100 g oil), β-sitosterol being the most abundant (76%–86% of total sterols). Low concentrations were recorded for campesterol, Δ5-avenasterol and cholesterol (11.2–48.7, 9.5–31.4 and 0.0–52.6 mg/100 g oil, respectively). For 24-methylene–cycloartanol, gramisterol, Δ7-stigmasterol, Δ7-avenasterol and citrostadienol were noted values below 10.2 mg/100 g oil.

Ramadan et al. [48] identified minor quantities (<35 mg/kg) of stigmasterol, D5, 24-stigmastanol, and D7-stigmastanol in cold pressed apricot kernel oil.
Stryezka et al. [40] showed that β-carotene content depends on the cultivar of the plant. The highest content of β-carotene was observed in the 'Somo' cultivar (66.8 μg/g oil), and the lowest in 'Goldrich Sungiant' (42.3 μg/g oil).

Apricot kernel oil extracted with SE had higher total phenolic content (26.9 μg gallic acid/g oil) than oil extracted with CP (24.9 μg/gallic acid/g oil) [40].
The content of polyphenols depended on the cultivar [42], the 'Somo' cultivar had the highest content of polyphenols (1.22 mM GAE/L), while 'Goldrich Sungiant' and 'Early Orange' had the least (0.85 and 0.87 mM GAE/L), respectively.

Table 4. Bioactive compounds present in apricot kernel oil

Method	Tocopherols (mg/kg oil)				Carotenoids (g/ 100 g oil)	Reference
	α	β	γ	δ		
CP	39.6	9.2	498.5	15.1	ND	[36]
SE	27.4	11.3	318.9	17.2		
CP	942				ND	[37]
SC	500					
SE	19.6-40.0	ND	315.4-502.3	28.3-58.5	42.3-66.8	[38]
SE	14.8-40.4	ND	330.8-520.8	28.5-60.2	ND	[41]
CP	/	/	550±0.2	19±0.0	ND	[46]

EXTRACTION OF OIL FROM PEACH KERNEL

Prunus persica is one of the species of the *Rosaceae* family that is widely distributed in most countries around the world. Peach is the second most important fruit crop in the European Union (EU) (approx. 3.8 million tons) after the apple [49]. The pulp from peaches is used directly for jams and canned food or diluted to prepare commercial or domestic

juices [50]. In addition, the leaves of the peach tree are used for the treatment of irritated digestive tract and constipation [51].

Table 5. Extraction of peach kernel oil

Extraction yield (%)	Method used for extraction	Fatty acid composition (%)							Location	Reference
		C18:1	C18:2	C18:3	C16:1	C16:0	C18:0	C20:0		
42.8	SE				ND				India	[35]
ND	SE	57.46	25.44	ND	ND	5.93	ND	6.18	Turkey	[45]
16-44	SE	52.5-72.9	18.51-26.1	0-0.06	0-0.30	6.0-14.8	2.25-5.1	0.05-0.15	Brazil	[54]
0.4-8.8	Mac	44.1-63	18-33.0	0-1.2	0-1	8.0-23	3.2-15.4	0-0.1		
0.17	HD	42.3	32.4	0	0	21.4	3.3	0		
3.80-24	SC-CO_2	67.6-79.7	15-19.90	0-1.1	0.21-0.9	5.9-9.2	2.26-2.6	0-0.2		
48	SE	74.6	15.7	0.1	0.5	6.0	2.1	0.2	Spain	[55]
30	SC-CO_2	72.6	17.7	0.0	0.6	6.6	1.9	0.1		
32	SC-CO_2 with ethanol	72.2	18.1	0.05	0.6	6.2	2.1	0.2		
Petroleum ether (g/g d.b) 0.25±0.04	SE	65.77 ±1.293	25.98 ±1.970	0.131 ±0.011	0.276 ±0.051	5.632 ±0.013	1.966 ±0.231	0.034 ±0.002	Canada	[56]
Chloroform 0.35±0.06		65.74 ±0.361	26.02 ±0.892	0.131 ±0.002	0.277 ±0.098	5.634 ±0.114	1.965 ±0.089	0.04 ±0.005		
Ethyl ether 0.38±0.07		65.76 ±2.034	25.89 ±0.099	0.132 ±0.007	0.280 ±0.016	5.691 ±0.086	1.983 ±0.007	0.034 ±0.006		
Hexane 0.26±0.04		61.87 ±0.068	29.07 ±0.013	0.161 ±0.000	0.252 ±0.001	6.355 ±0.035	1.917 ±0.011	0.038 ±0.003		
35.3	SC-CO_2				ND				Turkey	[57]
ND	SE	69.3	20.5	ND	0.4	6.1	1.9	0.0	Tunisia	[58]
ND	SE	59.8-64.6	27.9-32.8	0.31-0.42	0.53-0.69	4.13-5.82	1.25-1.44	ND	Pakistan	[59]
ND	SE	70.3	19.5	0.1	ND	5.9	1.6	0.1	Turkey	[60]

The kernel is considered an important food source with a high nutritional value, mostly due to its oil and protein contents [52] but they are usually destined to animal feed or used as fuel [53]. However, each year, thousands of tons of stones (pericarp plus kernel) from peaches are wasted as a by-product of the production of juices and jams.

Therefore, in the literature there are available papers which report extraction of oils from peach kernel [35], [45], [54]-[60] mostly with Soxhlet extraction using non-polar solvents. Few research groups have compared the conventional method with SC-CO_2 [54], [55].

In the study of Ferreira et al. [54] Soxhlet, maceration, hydro distillation and SC-CO_2 with different extraction solvents and key parameters in SC-CO_2 were tested. Among Soxhlet extractions, those carried out with DCM, EtAc and EtOH provided the highest yields, also the resulting extracts contained large variation in composition due to the broad range of polarity of solvents. The HD yield was the lowest value (0.17±0.02%), followed by Mac–EtAc, Mac–water and Mac–Hx (0.4±0.1%, 1.1±0.3% and 1.9±0.1%, respectively). The results for pure CO_2 indicated the maximum yield of 23.5 (0.4% (w/ w)) obtained at 50 ˚C/300 bar, with solvent density of 0.871 g CO_2/cm^3.

No differences in the composition of fatty acids profile were determined among different methods used, nevertheless higher yield of extraction was achieved with Soxhlet extraction in the study conducted by Pando et al. [55].

Higher yield of oil with ethyl ether was obtained in comparison with hexane, chloroform and petroleum ether with Soxhlet, although there was not observed a change in quantity of fatty acids [56].

Anwar et al. [59] showed that the fatty acids composition of kernel oils varies widely among different plant species.

These studies show that apricot kernel oil consists of more than 85% unsaturated fatty acids with the dominant oleic acid, besides palmitic and stearic acid being the main saturated fatty acids with amounts less than 10% (table 5).

BIOACTIVE COMPOUNDS IN PEACH KERNEL OIL

Pando et al. [55] with superfluid extraction determined γ-tocopherol and γ-tocotrienol; their contents in the oil extracted were 44 and 150 mg/kg, respectively. The other forms of tocopherol and tocotrienol were not detected.

In the study of Anwar et al. [56] quantity of tocopherols varied among different plant species. The corresponding contents of α-tocopherol, δ-tocopherol and γ- tocopherol in peach kernel oil ranged from 175.4-187.5, 74.5-85.9 and 110.2-126.7 mg/kg, respectively.

In the study of Ozcan and Mathaus [57] α-tocopherol was the dominating tocopherol with 37.3 mg/kg, followed by γ-tocopherol with 1.6 mg/kg. Also, α-tocotrienol was present in peach kernel oil with amount 24.0 mg/kg.

The amount β-sitosterol extracted from peach kernels was 1220 mg/kg kernel at optimal values of 40 °C, 200 bar, 7 ml/min, 0.3 mm and 3 hours [54].

The main phytosterol component in peach kernel oil was established to be β-sitosterol amounting 78.8-80.0% followed by Δ5-avenasterol with levels 8.9-12.2%. A considerable amount of campesterol and Δ7-avenasterol within the range of 4.1 to 5.9% was detected in the tested oils [56].

Total phenolic compounds were determined 128±5 mg GAE/g from SE-EtOH sample. Otherwise, the mixture (EtOH/water) in Soxhlet extraction resulted in a poor solvent for phenolic compounds, with TPC of 0.3±0.1 mg GAE/g. High TPC values were also obtained by Mac–EtOH and Mac–Hx fractions, 83±3 mg GAE/g and 93±5 mg GAE/g, respectively. In the SC-CO_2 there was a trend to increase the TPC with pressure. The lowest TPC value was observed at 100 bar and 50 ˚C (0.18±0.07 mg GAE/g), while the highest TPCs were obtained at 300 bar for all temperatures and at 200 bar and 40 ˚C (31±2 mg GAE/g) [51].

The oils extracted with solvents in the study by Wu et al. [53] resulted in low phenolic contents (3.829-4.1593 mg GAE/g). Although the polarities of chloroform and ethyl ether were stronger, hexane provided the higher extraction efficiency than other solvents. Rutin was the predominant phenolic compound in the oil extracted with hexane accounting for 76.65 g/100 g of the total amount.

Table 6. Bioactive compounds present in peach kernel oil

Method	Tocopherols (mg/kg oil)				Sterols (%)			Carotenoids (mg/ 100 g oil)	Reference
	α	β	γ	δ	β-sitosterol	Campesterol	D5-avenasterol		
SC-CO_2	/	/	44	/	ND	ND	ND	ND	[52]
SC-CO_2	ND	ND	ND	ND	1220 mg/kg seed	ND	ND	ND	[54]
SE	175.4-187.5	ND	110.2-126.7	74.5-85.9	78.83-80.01	4.13-4.39	8.90-12.18	ND	[56]
SE	37.3	ND	1.6	ND	ND	ND	ND	ND	[57]

EXTRACTION OF OILS FROM OLIVE KERNELS

The Olive (*Olea europaea* L.) is a small tree, which belongs to the family *Oleaceae* and is native to tropical and warm temperate regions of the world. The tree, famous for its fruit, is commercially important in the Mediterranean region as a prime source of olive oil [61]. Olive oil is widely used for food preparations and as a result of olive processing, a huge quantity of olive by-products are produced. The olive stone and seed are important by-products generated in the olive oil extraction, the whole olive stone is a rich source of bioactive compounds. These potentially valuable compounds are nuzhenide-oleoside, nuzhenide, salidroside, which are detected only in the olive seed; verbascoside only appears in significant quantities in the seed and pulp [62].

Alves et al. [63] extracted lipids from olive seeds at two ripeness stages (green and ripe). In both olive seeds, the predominant FA are C18:1 (56%), C18:2 (17%), and C16:0 (18%). In total lipid extract, ripeness caused a shift in the FA profile promoting an increase of 5.46% in C18:1 and a decrease in the rest of FA, except for C16:1 whose relative abundance was the same in both stages.

Lipids extraction using chloroform:methanol (2:1) from olive seeds [64] resulted in oil rich in oleic

(59.9%) and linoleic acid (16.3%) followed by palmitic acid (15.5%). The total lipids obtained in this study were 74.2±5.6.

Ranalli et al. [65] extracted lipids from seeds of seven olive varieties grown in Italy. The extraction was performed on Soxhlet apparatus using petroleum ether as solvent. The oil extracted had high content of unsaturated FA (85.38%) and unsaturated FA (13.53%).

Table 7. Extraction of oil from olive kernels

Extraction yield (%)	Method used for extraction	Fatty acid composition (%)							Location	Ref.
		C18:1	C18:2	C18:3	C16:1	C16:0	C18:0	C20:0		
ND	Mac Ripe	59.07 ±1.68	17.15 ±0.42	0.10 ±0.05	ND	16.04 ±0.64	6.81 ±1.04	0.33 ±0.11	Portugal	[63]
	Green	53.61 ±0.97	17.25 ±0.38	0.12 ±0.02	ND	17.61 ±1.43	10.22 ±0.69	0.45 ±0.11		
74.2 ±5.6	SE	59.9	16.3	0.1	ND	15.5	7.5	ND	Portugal	[64]
ND	SE	68.02 ±6.37	16.55 ±1.84	0.42 ±0.04	0.39 ±0.04	10.15 ±1.02	2.87 ±0.24	0.51 ±0.05	Italy	[65]

APPLICATION OF FRUIT KERNEL OILS

Oils derived from edible vegetables, fruits, seeds, tree and ground nuts have been safely consumed by, and applied to the skin of humans for thousands of years.

COSMETICS

Dermal toxicity of sour cherry kernel oil was tested on guinea-pigs for 21 days which resulted in none of the tested animals to exhibit adverse changes to the skin suggestive of an allergic or otherwise toxic reaction to contact with oil. The protection against UV damage had shown that creams containing this kernel oil at a dosage of 3% or greater provided significant protection [66].

Cosmetics companies report SCKO as it helps to reduce the signs of fine lines and wrinkles. It moisturizes the skin and can be used for hair and nail care as well [67], [68].

Apricot kernel oil (0.005%) resulted in not a dermal irritant or sensitizer when applied neat in a scalp/hair wax conditioner tested in total of 104 people [69].

A test was conducted in which participated 108 people by applying 19.749% apricot kernel oil in a face serum in this case eczema and erythema were observed [70]. Same results had appeared when 2.5% apricot kernel oil was applied in cream in which participated 119 people [71]. Although, in both these studies AKO was classified as not a primary irritant.

Interestingly, in a face cream (HRIPT-51) and eye cream (HRIPT-108) with 2% apricot kernel oil with 20 μL test material occluded it was shown that AKO is not a dermal irritant or sensitizer [72], [73]. The same was proven for a cream containing 1% apricot kernel oil applied neat on finn chmabers (HRIPT-57) [74].

Cosmetics companies report AKO as an extraordinarily versatile and skin-friendly base oil which is quickly absorbed and ideally suited for mature and sensitive skin, softens the skin and gives a radiant complexion [75], [76].

24% peach kernel oil in a lip balm was tested on 222 people with 0.2 g material occluded; 2 participants had low level, transient reactions during the induction, no other reactions were observed. This study concluded that test material was not a dermal sensitizer [77].

Cosmetic companies report PKO as oil with light texture that absorbs quickly without leaving a sticky film. It protects sensitive, dry, and mature skin and can help to strengthen the skin's immune system. Peach kernel oil smooths and hydrates the skin, improves skin elasticity and leaves a soft and supple feel [78], [79].

PHARMACEUTICALS

A microemulsion of sour cherry kernel oil was orally administered to mice at doses of 2.5%, 5%, and 10% for 10 days. In this case there was not toxicity evidence of this product in the dose range used in foods or healthcare, but also it improved the cardiac function recovery of the tested animals [80].

SCKO was tested for its antimicrobial activity in a study conducted by Kazempour-Samak et al. [81] in

which it was shown that it inhibited the growth of all microbial species tested especially Gram-positive strains. The most sensitive microorganism (lowest MIC) among the studied microorganisms was *Listeria monocytogenes*.

SCKO loaded gum Arabic and Maltodextrin microcapsules developed by spray-drying technique displayed antimicrobial activity against all pathogenic bacteria tested except *Escheria coli* when evaluated by agar well-diffusion assay, while the greatest antimicrobial activity was observed against *Pseudomonas aeruginosa* [82].

SCKO nanoemulsion was tested for cytotoxic impacts and apoptotic activity, anti-tumour effect by Maragheh et al. [83]. The results indicated the 36.5 nm stable SCKO-NE significantly decreased the breast cancer line MCF7 cells viability comparing with normal Human foreskin fibroblasts (HFF) cells and reduced the tumor size.

Nano-emulsions and nano-emulgels containg 2% statins and 8% apricot kernel oil were formulated and tested as alternative delivery system of statins [84]. Membrane release studies indicated that statins were released at higher flux values in nano-emulsions compared to their respective nano-emulgels. *Ex vivo* (skin diffusion) studies indicated higher median values in the nano-emulgels compared to their nano-emulsion counterparts.

In a rat model of chemically induced (trinitrobenzene sulfonic acid) ulcerative colitis, Minaiyan et al. [85] have used apricot extract and extract/oil and compared it with a standard treatment (prednisolone). The authors reported that both, on the macroscopic and microscopic levels, showed a significant improvement in disease activity.

Apricot kernel oil protects rat gastric mucosa against ethanol induced injury [86]. Group of albino rats treated AKO+ethanol exhibited significantly fewer gastric lesions compared to the ethanol group.

NUTRACEUTICALS

Apricot kernel oil (1.0%) was incorporated in chitosan films used for package of spiced beef by Wang et al. [87]. The results of this study indicated that CS films with AKO had better sensory attributes including taste, color, texture and overall acceptance during the whole storage period. Also, it was shown that it can display antimicrobial effects against *Listeria monocytogenes*.

AKO decreased markedly the cell line viability and migration of carcinoma of the tongue HNO97. Apricot oil caused no significant inhibition of normal oral epithelial cells viability in low doses. Shalash et al. [88] proved that AKO can be used as nutraceuticals in the treatment of oral cancer.

The effects of dietary apricot kernel oil (AKO) were evaluated in a rat model of cyclophosphamide-induced immunosuppression in the research of Tian et al. [89]. Rats had intraperitoneal injection with cyclophosphamide to induce immunosuppression and were then infused with AKO or normal saline (NS) for 4 weeks. Compared to the normal saline-treated group, lymphocytes isolated from rats administered AKO showed significant improvement in immunoglobulin IgA, IgM, IgG, interleukin (IL)-2, IL-12, and tumor necrosis factor-α (TNF-α) and reduced oxidative stress in rats treated with AKO. Dietary AKO positively affected rat growth and inhibited cyclophosphamide-associated organ degeneration. Thus, the use of AKO as a nutritional supplement can be proposed to ameliorate chemotherapy-associated immunesuppression.

Hao et al. [90] showed that peach kernel oil could reduce total cholesterol, triglyceride, low-density lipoprotein cholesterol levels, elevate the high-density lipoprotein cholesterol level in serum, and reduce the area of the aortic atherosclerotic lesions in high-fat diet fed Apolipoprotein E knockout mice. Moreover, peach kernel oil treatment resulted in significantly down regulate the expression of TF protein to inhibit the formation of atherosclerotic plaque. This study proves peach kernel oil may be a potential health food to prevent atherosclerosis in cardiovascular diseases.

CONCLUSION

In this review were highlighted the most prevalent data regarding extraction of oil from kernels of sour cherry, apricot, peach and olive and the different method used. Fatty acid composition and the presence of bioactive constituents in the oils was analyzed. A lot of research groups reported oil extraction from sour cherry, apricot and peach and only a few publications were available for olive kernels. All the authors report that these oils are very rich in poly unsaturated fatty acids, the predominant oleic acid followed by linoleic acid which makes them very attractive for their appliance in various industries. Many bioactive constituents were identified and quantified as tocopherols, tocotrienols, phytosterols, carotenoids and phenolic compounds.

Both *in vitro* and *in vivo* studies of sour cherry, apricot and peach kernel oil showed that they possess antioxidant, anti-microbial, apoptotic and anti-tumor activities. Clinical trials on humans of cosmetics containing these oils show that they are not a dermal irritant or sensitizer.

ABBREVIATIONS

ND-no data
C18:1-oleic acid
C18:2-linoleic acid
C18:3-linolenic acid
C16:1-palmitoleic acid
C16:0-palmitic acid
C18:0-stearic acid
C20:0-arachidic acid
SE- Soxhlet extraction
CP- cold press
SCKO-sour cherry kernel oil
GAE-gallic acid equivalent
GA-gallic acid
AKO-apricot kernel oil
DCM-dichloromethane
EtAC-ethyl acetate
EtOH-ethanol
HD-hydro distillation
Mac-maceration
HX-hexane
TPC-total phenolic compound
PKO-peach kernel oil
HRIPT-Human Repeat Insult Patch Test
MIC-minimum inhibitory concetration
CS-chitosan

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INVESTIGATION OF THE CATALYTIC ACTIVITY OF HYDRATED LIME $Ca(OH)_2$ IN THE PROCESS OF TRANSESTERIFICATION OF VEGETABLE OILS

ORIGINAL SCIENTIFIC PAPER

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DOI: 10.51558/2232-7568.2023.17.1.29

RECEIVED 2024-02-21 **ACCEPTED** 2024-04-11

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ABSTRACT:
Currently, humanity is facing two existential problems: the constant reduction of fossil fuel supplies, primarily crude oil, and global climate change, which is a direct consequence of the increasing use of fossil fuels both in industry and in the transport sector [1, 2]. One of the possible solutions for these problems are biofuels, fuels obtained from renewable raw materials, as it is biodiesel [2], which attracted attention due to characteristics such as high degradability, non-toxicity and low emission of carbon monoxide, particulate matter and unburned hydrocarbons, as well as the possibility of being used either in a mixture with fossil with diesel or independently as 100% biodiesel fuel [3, 4, 5, 6]. Heterogeneous catalysts in transesterification processes, i.e. biodiesel production, have been an area of significant and extensive research for many years. It is noticeable that there are significantly fewer works in which the application of $Ca(OH)_2$, was investigated, and the published works show conflicting results, both in terms of its catalytic activity and in terms of the achieved yield of fatty acid methyl esters (FAME). The main goal of this work was to analyze the physico-chemical, chemical, mineralogical, morphological and surface characteristics of hydrated lime produced by Stamal Ltd. Kreševo, with the aim of examining the possibility of its application as a catalyst in the process of transesterification of vegetable oils. The obtained results unequivocally show that by using this hydrated lime as a catalyst in the transesterification process of rapeseed oil, it is possible to achieve a yield of methyl esters that meets the minimum limit of 96.5% prescribed by the European standard for biodiesel, EN 14214.

KEYWORDS: biodiesel, heterogeneous catalysts, hydrated lime

INTRODUCTION

Biodiesel is a liquid biofuel that consists of monoalkyl esters of vegetable oils, animal fats, or other lipid raw materials such as used cooking oil. Due to its physicochemical properties similar to conventional diesel, biodiesel can be used in unmodified compression ignition engines, either in its pure form (designated as B100), or in blends with petroleum diesel [7, 8, 9] where it further improves lubricity and increases fuel cetane number. Among the existing methods of biodiesel production, the conventionally applied method is the transesterification of vegetable oils with alcohol, typically methanol (so-called methanolysis) in the presence of a catalyst, which converts oil triglycerides into alkyl esters, whereby biodiesel is produced as the main product and crude glycerol as a by-product [10, 11].

Although the transesterification reaction that produces biodiesel from triacylglycerol raw materials is balanced and the transformation basically occurs by mixing the reactants, the use of a catalyst increases the solubility of alcohol in oil and thereby accelerates the reaction [12], which enables the reaction to take place at low and moderate temperatures. The main process conditions whose optimization is of great importance for the efficient production of biodiesel include: molar ratio of alcohol to oil, type and amount of catalyst, temperature and reaction time [13, 14].

Homogeneously catalyzed transesterification reactions, which are dominant in industrial practice today, are typically fast and require a smaller amount of catalyst than heterogeneously catalyzed ones [15]. In general, it can be said, and this is confirmed by published research, that alkaline-catalyzed reactions take place much faster than acid-catalyzed ones. Kinetic coefficients for alkaline catalysts were two to

four orders of magnitude higher than those of acidic catalysts [16]. In addition, a very important feature of alkaline catalysts is that they are significantly less corrosive to industrial equipment than acidic ones, so it is not surprising that commercial biodiesel synthesis processes on an industrial scale are usually carried out with highly alkaline homogeneous catalysts, such as sodium hydroxide (NaOH), potassium hydroxide (KOH), sodium methoxide ($NaOCH_3$) and potassium methoxide ($KOCH_3$) [17, 18, 19, 20, 21]. The introduction of heterogeneous catalysts into the commercial process of biodiesel production could reduce its cost and make it competitive with fossil diesel, given the number of advantages these catalysts offer, including higher reaction rates, easy separation from products, and reusability [22]. Heterogeneous catalysts in transesterification processes, i.e. biodiesel production, have been an area of significant and extensive research for many years.

Table 1. Review of literature references in which $Ca(OH)_2$ was used as a catalyst for transesterification of various vegetable oils

Catalyst type	Raw material (oil)	T (°C)	Methanol/oil ratio	Catalyst (wt.%)	Time (min)	Biodiesel yield (wt.%)	Reference
$Ca(OH)_2$	Oilseed rape	65	0,225 mol/50 cm^3	0,0075 mol	150	/	[25]
$Ca(OH)_2$	Soy	65	12:1 mol	14 mmol	60	12	[26]
$Ca(OH)_2$	Sunflower	50	12:1 mol	Equivalent to 1% NaOH	1440	<70	[27]
$Ca(OH)_2$	Soy	300	39,4:1 mol	0,68	10	98	[28]
$Ca(OH)_2$	Soy	70	9:1 mol (ethanol)	13,8	600	<15	[29]
$Ca(OH)_2$	Sunflower	60	6:1 mol	5	120	>90	[30
$Ca(OH)_2$	Barbados nut	65	20 wt% CH_3OH	1,5	60	76,75	[31]
$Ca(OH)_2$	Peanuts (raw oil)	78	9:1 mol (ethanol)	1,0	180	8,08	[33]
$Ca(OH)_2$	Peanuts (raw oil)	100	12:1 mol (ethanol)	2,0	120	<10	[32]
$Ca(OH)_2$	Sunflower	60	6:1 mol	1,5	60	<20	[34]
$Ca(OH)_2$	Soy	60	50 vol.% CH_3OH	3,8	120	100	[35]
$Ca(OH)_2$	Castor oil	60	51 vol.% CH_3OH	1,6	120	93,5	[36]
$Ca(OH)_2$	Oilseed rape	60	12:1 mol	5	180	>90	[37]
$Ca(OH)_2$	Sunflower	60	25 vol.% CH_3OH	4	120	93,23	[24]
Ca(OH)2/KF	Palm oil	65	12:1 mol	5	1	97,6	[38]

There is a significant number of published results of the catalytic activity of different types of catalysts, where there are significant differences regarding the optimal process conditions, and in some cases also regarding the existence of the catalytic activity of certain compounds, which generally depends on their nature, size and specific surface area of the particles, and the applied reaction conditions [23, 24]. Given that Bosnia and Herzegovina has a significant number of limestone deposits of different composition and several production capacities of $Ca(OH)_2$, the application of hydrated lime as a catalyst for obtaining biodiesel could economically significantly improve both the industry itself related to the production of limestone-based products (by creating new market for products), as well as the industrial production of biodiesel (lower-price catalysts, less environmental burden), which would further result in market, environmental and numerous other advantages.

The largest number of studies on methanolysis with the use of calcium-based catalysts refers to the application of calcium oxide [24], due to its low cost, good catalytic properties and the possibility of recycling without a major reduction in catalytic activity [23]. On the other hand, there is a relatively small number of researches based on the application

of $Ca(OH)_2$ as a catalyst, for the reason that for a long time it was considered that it has no significant catalytic activity in the process of transesterification of vegetable oils.

A literature review of references in which the influence of $Ca(OH)_2$ as a catalyst in the process of transesterification of various vegetable oils was investigated is given in table 1.

MATERIAL AND METHODS

MATERIAL

The following materials were used in the experimental part of the research:
- commercial hydrated lime (Stamal Ltd. Kreševo),
- refined rapeseed oil (Bimal Ltd. Brčko),
- methanol p.a. (Fluka),
- other materials and chemicals needed to carry out the necessary analyses.

METHODS

Determination of structural properties of the catalyst was done using the X-ray diffraction (XRD) method. The diffractograms of the samples were recorded on an automatic X-ray powder diffractometer Philips PW1710, at an operating voltage of U = 40kV and a current of I = 30mA. CuKα radiation with a wavelength of λ = 1.54056 Å was used, monochromatized using a graphite monochromator. Before starting the recording of the samples, the accuracy of the diffractometer device was controlled using the basic program PW-1844. The step size was 0.02º 2θ with a time delay of 1 second at each step. The angular recording interval was 2-80º 2θ. Diffraction data were collected at room temperature. Based on the obtained values of intensity I (imp) and interplanar distances d (Å), by comparison with literature data and ICDD standards, identification was made.

The determination of the chemical composition of the catalyst was carried out using the method of X-ray fluorescence analysis (XRF) on a Bruker S8 Tiger XRF spectrometer, at the operating voltage of the spectrometer X-ray tube 40 kV, the maximum current 10 mA and the maximum power of the X-ray tube 400 W.

The catalyst composition was determined by thermal analysis methods (TG/DTG), using a Q600 thermal analyzer (TA Instruments, New Castle, DE, USA). Analyzes were performed in a corundum cone, in a nitrogen atmosphere at a flow rate of 100 cm^3/min and at a heating rate of 20°C/min. The mass of the sample by measurement was 2-3 mg. The reference sample was an empty cone. The temperature interval of the analysis ranged from room temperature to 1000°C.

The determination of the morphological properties of the catalyst was performed using scanning electron microscopy with energy-dispersive spectrometry (SEM-EDS) on a JEOL JSM 6420 LV device at an accelerating voltage of 20 kV. Catalyst samples were prepared for analysis by being coated with a 15 nm thick layer of gold with a density of 19.32 g/cm^3. The element used for optimization is nickel.

The textural properties of the catalyst (specific surface area, mean pore diameter and total pore volume) were determined by the Brunauer–Emmett–Teller (BET) method. Sample preparation for analysis was done by activation at 400°C for 2 hours, followed by degassing in a nitrogen atmosphere at 400°C for 1 hour. The device on which the analysis was performed is Micromeritics - Gemini VII, Version 5.0, Model 2390, and the measurement was based on the static monomolecular adsorption of nitrogen N_2 at the temperature of its liquid aggregate state (-196ºC) in the carrier gas stream.

A laser diffraction particle size analyzer, Malvern Mastersizer 2000, was used to determine the granulometric composition of the catalyst. The device works on the principle of diffraction of red and blue laser light on particles in dispersions. The system consists of a Mastersizer 2000 optical system and a Hydro 2000 dispersion unit for the characterization of dispersions in liquid dispersants and a computer with installed software.

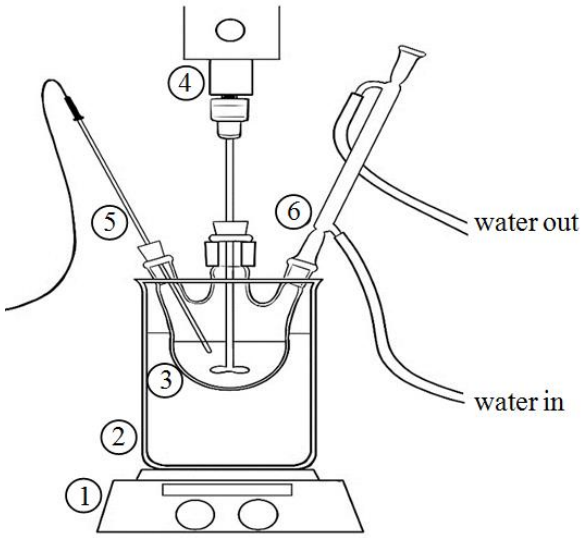

Figure 1. Laboratory apparatus for obtaining biodiesel

The apparatus that was used to carry out the heterogeneous-catalyzed methanolysis of rapeseed oil (Figure 1) consisted of the following components:

1. Electric heater with thermostat,
2. Glass cup,
3. Three-necked flask with a volume of 500 cm^3 with a round bottom,
4. Mechanical mixer,
5. Temperature probe i
6. Water cooler

To test the influence of the amount of added catalyst on the transesterification of rapeseed oil, that is, on the yield of FAME and the basic characteristics of the obtained biodiesel sample, catalyst concentrations (wt.%) of 2, 3, 4 and 5 were used in relation to the mass of rapeseed oil. The reaction time was 120 min, and the temperature at which the reaction was carried out was 60°C. A methanol/oil volume ratio of 0.25 was used in all experiments. The mixing was more intense at the beginning of the experiments, considering the nature of the ingredients in the initial mixture, and after homogenization, the mixing was constant and amounted to 1000 rpm. After the expiry of the set methanolysis time, the liquid phase was separated from the catalyst by a vacuum pump and left for 24 h in a funnel to separate the fractions of biodiesel (methyl ester) and glycerol based on their different specific gravity. Then the lower (glycerol) phase was discharged from the funnel, and the separation of fine catalyst particles and residual glycerol from the biodiesel fraction was performed by centrifugation at 3000 rpm (Eppendorf Centrifuge 5702) for 10 min at room temperature. The biodiesel thus obtained was further analyzed for fatty acid methyl esters (FAME) content.

Determination of FAME content was performed on a gas chromatograph 7890A with FID detector and automatic sampler 7683B, manufactured by Agilent Technologies, according to the standard SRPS EN 14103, April 2008, identical to EN 14103:2003. The aforementioned standard defines the determination of the ester content in fatty acid methyl esters intended for use as pure biofuel or as a component for mixing fuel oil and diesel fuel.

RESULTS

The morphology of the surface of the hydrated lime produced by Stamal, used as a catalyst, is presented in Figure 2. SEM photographs show an uneven morphology in the form of aggregates consisting of irregularly stacked $Ca(OH)_2$ crystals of different sizes and shapes, resulting in "open" structure of the material.

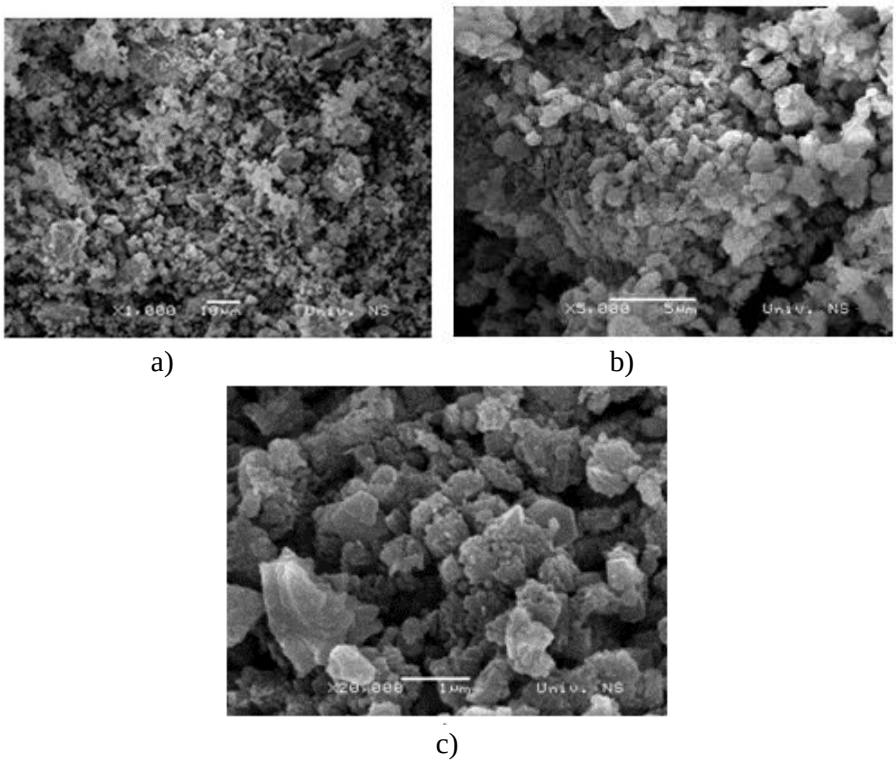

Figure 2. SEM photos of unused hydrated lime produced by Stamal, at magnifications of: a) 1000, b) 5000, c) 20000

The textural parameters of hydrated lime are as follows: the sample has an average pore diameter of 2.0 (nm) and pore volume (0.007 cm^3/g), and therefore a relatively small specific surface (11.01 m^2/g).

Energy dispersive X-ray spectroscopy (EDS), which was performed in combination with SEM, shows the elemental composition (wt.%) of the lime sample (Fig. 3): 39.07 Ca, 47.68 O, 11.56 C, 0.51 Mg, 0.39 Al, 0.56 Si and 0.24 Fe. Carbon, which is registered in the sample in a slightly larger amount, can also be an indication of the presence of a carbonate phase ($CaCO_3$).

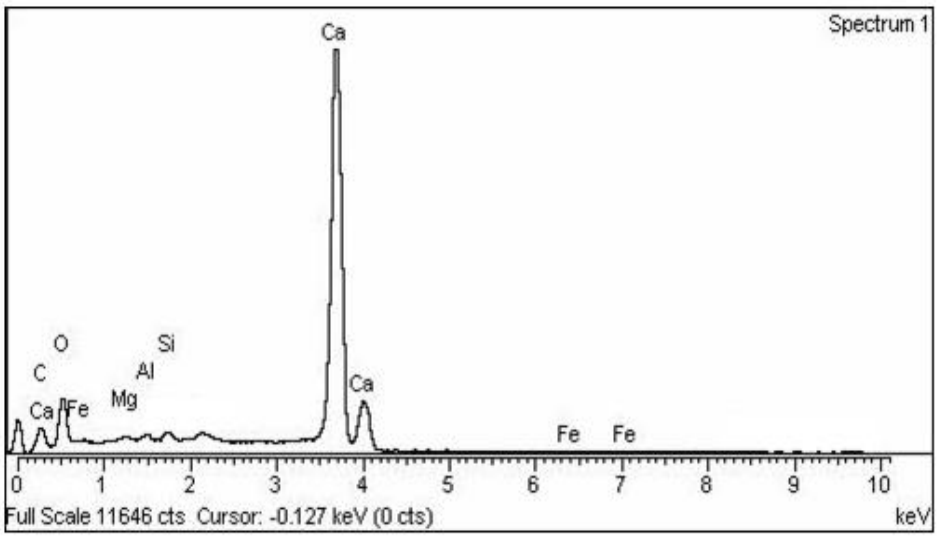

Figure 3. EDS spectrum of Stamal hydrated lime

Stamal hydrated lime particles have a narrow size range (1–80 μm) which is shown in Figure 4. In addition, they can be characterized by a bimodal distribution of particle sizes, where a slightly larger part of the particles is in the 11–70 μm range, and the rest in the 1 -10 μm. About 10 vol.% of the particles have a diameter smaller than 2.56 μm, about 50 vol.% of the particles are smaller than 9.926 μm, while 90 vol.% of them are characterized by a diameter below 34.6 μm. The average diameter of the particles is 14.73 μm.

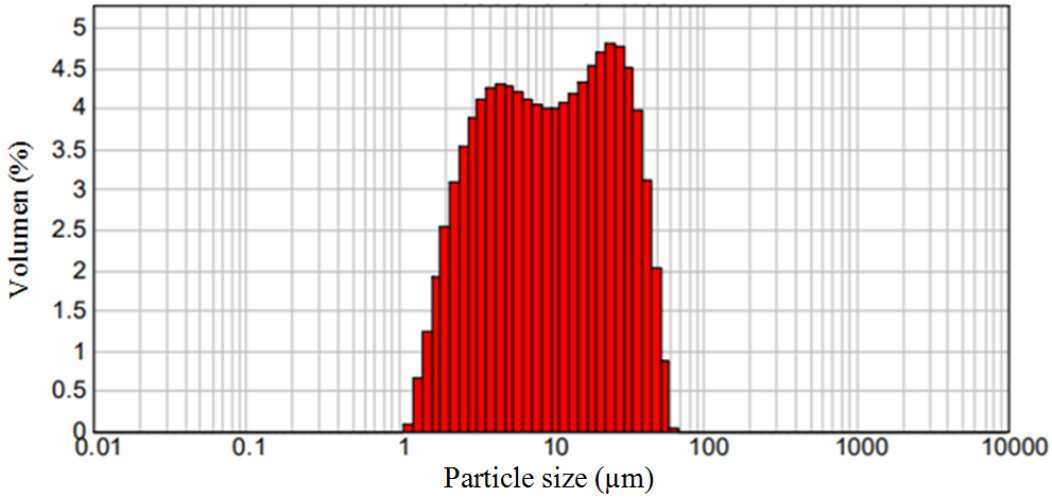

Figure 4. Particle size distribution of Stamal hydrated lime

The results of the XRF analysis of Stamal hydrated lime, which show its chemical composition in the form of oxides, are shown in Table 2. The largest part of the sample is CaO (63.63 wt.%), while SiO_2 was detected in a much lower concentration (1.58 wt.%), and other elements are present in very small amounts (<1.00 wt.%). The resulting loss on ignition (LOI) is related to the removed water and CO_2 from $Ca(OH)_2$ and $CaCO_3$ after exposing the sample to high temperature.

Table 2. Chemical composition of hydrated lime produced by Stamal Ltd. Kreševo

Chemical composition	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	SO_3	Na_2O	K_2O	MnO	TiO_2	P_2O_5	LOI	Sum
wt.%	1.58	0.68	0.44	63.63	0.97	0.51	0.016	0.075	0.018	0.017	0.009	31.71	99.66

The XRD diagram of Stamal hydrated lime is given in Figure 5. The diffractogram shows two components of the catalyst: portlandite – $Ca(OH)_2$, with the two most intense peaks around 2Θ values of 18° and 34°, and calcite – $CaCO_3$, with the most intense peak at 2Θ values between 29° and 30°. Given that calcite was identified by this method, the earlier assumption from EDS results about its presence can be confirmed. According to the XRD results of the quantitative analysis, the sample contains 89.63 wt.% portlandite, which corresponds to 67.83 wt.% CaO, and this is in good agreement with the XRF results.

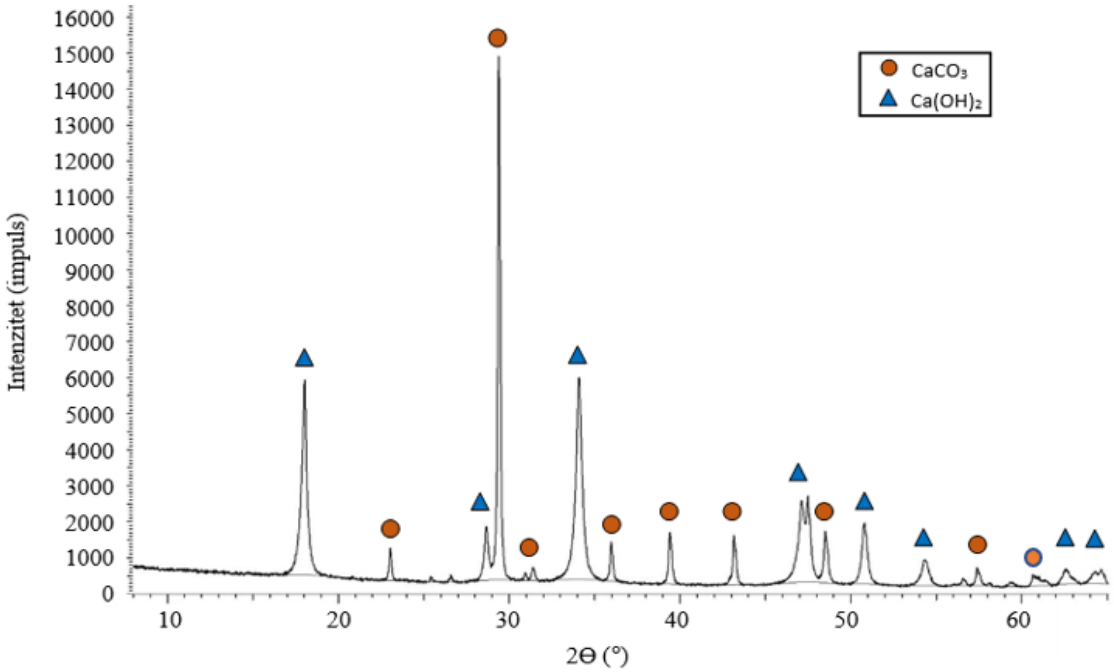

Figure 5. XRD diagram of Stamal hydrated lime

Thermochemical changes of Stamal hydrated lime, which were examined by TG/DTG analysis, i.e. the weight loss profile when heated in a stream of nitrogen (N_2) is shown in Figure 6.

The weight loss of 0.7% in the temperature interval up to 300°C can be attributed to the loss of adsorbed water, which the Stamal sample contained in a relatively small amount.

Upon further heating to 500°C, the weight of the sample decreased by an additional 11.2%, which was caused by the dehydroxylation of $Ca(OH)_2$. In the temperature interval 500-770°C, the weight of the sample decreased by an additional 18.8%, as a result of the decomposition of the present $CaCO_3$ into CaO and CO_2, which is in accordance with other characterization results presented previously.

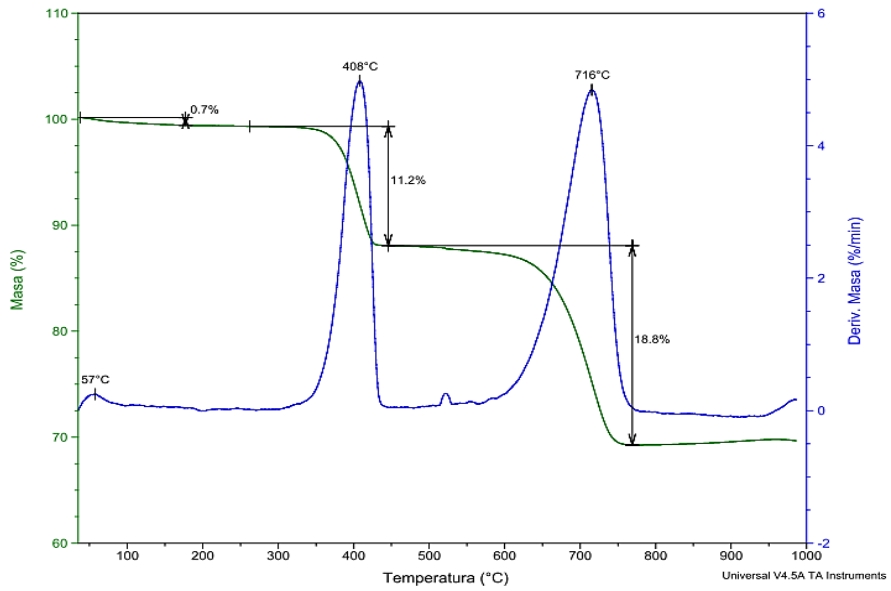

Figure 6. TG/DTG profile of Stamal hydrated lime

Figure 7 shows a diagram of the influence of the amount of catalyst (Stamal hydrated lime) on the yield of methyl esters, by carrying out the transesterification of refined rapeseed oil.

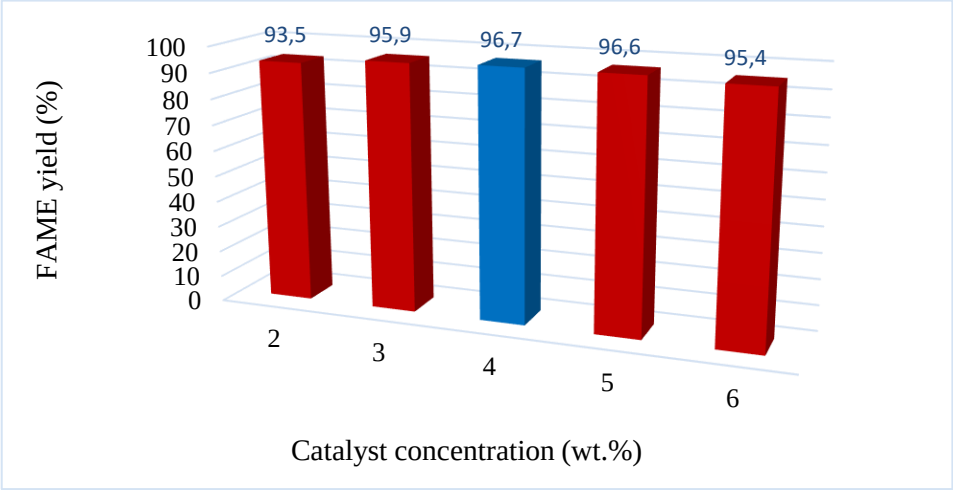

Figure 7. **Influence** of Stamal catalyst concentration on FAME yield

By using Stamal hydrated lime in a concentration of 2 wt.%, a yield of methyl esters of rapeseed oil of 93.5% was achieved. Although the FAME yield achieved is significant, the applied catalyst concentration did not yield a yield of rapeseed oil methyl esters that meets the minimum limit of the European standard EN 14214 of 96.5% [39]. This yield can be explained by the insufficient number of active sites for the reaction of reactants [40], i.e. relatively small specific surface area of the catalyst. Increasing the concentration of hydrated Stamal lime from 2 to 3 wt.% led to an increase in the yield of MEMK, which after a reaction time of 120 minutes was 95.9%. This yield at a catalyst concentration of 3 wt.% is due to the relatively low content of $Ca(OH)_2$ determined by XRD analysis (Fig. 5) and TG/DTG analysis (Fig. 6), i.e. due to the highest content of calcium carbonate ($CaCO_3$) which were shown by TG/DTG analysis (Figure 6) and XRF analysis (Table 2). Calcium carbonate has been shown to have very little or no catalytic activity, i.e. much lower than calcium hydroxide [26]. By increasing the concentration of Stamal hydrated lime to 4 wt.%, the yield of rapeseed oil methyl esters increased slightly and reached its maximum (96.7%), and by further increasing the catalyst concentration to 5 and 6 wt.%,

the FAME yield gradually decreased to 96 .6% and 95.4%.

CONCLUSION

Obtaining biodiesel by transesterification of rapeseed oil with the use of hydrated lime produced by Stamal Ltd. Kreševo, without prior activation of the catalyst, is possible to achieve a yield of methyl esters that meet the minimum limit of 96.5% prescribed by the European standard for biodiesel, EN 14214.

Under the conditions of a volume ratio of methanol to oil of 0.25, a temperature of 60°C, a duration of the transesterification reaction of 120 minutes, a catalyst concentration of 4 wt.% and a mixing speed of 1000 rpm, by a batch process of methanolysis with Stamal hydrated lime, as heterogeneous catalyst, the maximum yield of rapeseed oil methyl esters of 96.7% was achieved. The content of calcium hydroxide in hydrated lime is correlated with its catalytic ability in the transesterification reaction.

The content of $Ca(OH)_2$ in the sample of hydrated lime produced by Stamal is average when it comes to products of this type and amounted to 89.63 wt.% for the tested sample, and the maximum yield of biodiesel obtained under the mentioned transesterification conditions was 96.7%. The presence of a crystalline phase of calcium carbonate in hydrated lime is associated with a lower catalytic activity of lime, which additionally depends on the content and location of $CaCO_3$ in the catalyst structure. Given that a significant content of calcium carbonate and an average content of $Ca(OH)_2$ was found in the examined sample of hydrated lime, the catalytic activity of this lime is moderate.

The specific surface of Stamal hydrated lime of 11.4 (m^2/g) can also be considered as one of the reasons why this lime in the observed reaction conditions showed moderate catalytic activity, considering that to achieve a significant conversion of fatty acids into their methyl esters of 96,7%, it was necessary to use a catalyst in a concentration of 4 wt.%. From the obtained results it is evident that the concentration of hydrated lime as a catalyst can be taken as a very important factor on which the efficiency of the transesterification of vegetable oils with the aim of obtaining biodiesel depends. Lower concentrations will not ensure the smooth progress of the transesterification reaction, because not enough active sites will be provided. On the other hand, excessive catalyst concentrations can cause a decrease in the conversion of triacylglycerols into their methyl esters because they usually lead to an increase in the viscosity of the reaction mixture, which leads to difficult mass transfer.

The lowest yield of methyl esters (93.5%) was achieved when the catalyst was used in a concentration of 2 wt.%. By increasing the concentration of the catalyst to 3 wt.%, an increase in the yield of methyl esters to 95.9% was recorded, so that the maximum value of the yield of FAME of 96.7%, which is in accordance with the minimum requirement of the European standard EN 14214 for biodiesel, was achieved when the catalyst was used in amount of 4 wt.%. A further increase in the catalyst concentration (5 wt.% and 6 wt.%), led to a partial decrease in the yield of methyl esters (96.6% and 95.4%) from the previously achieved maximum (96.7%).

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OPTIMIZATION OF LIQUID SOAP FORMULATION

ORIGINAL SCIENTIFIC PAPER

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DOI: 10.51558/2232-7568.2023.17.1.49

RECEIVED 2024-05-10 **ACCEPTED** 2024-07-11

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ABSTRACT:
The quality of liquid soap for washing hands depends on its chemical composition, and it is evaluated by the physicochemical and functional characteristics of that product. The aim of the work is to prepare different series of liquid soap formulations by varying the concentrations of anionic and amphoteric surfactants and sodium chloride, and to optimize the formulation. The effects of the optimization were evaluated based on the results of the analysis of the physico-chemical parameters of the prepared formulations, such as density, viscosity, surface tension and critical concentration of micelles. As amphoteric and anionic surfactant concentrations in liquid soap increase, density and viscosity values increase. The value of the surface tension decreases with the increase in the concentration of the surfactants present, with the amphoteric surfactant making a greater contribution to the decrease. Regardless of certain advantages that the anionic surfactant shows in relation to the amphoteric one, the best characteristics of the liquid soap are shown by the formulation that contains a combination of both used surfactants. The addition of NaCl to the liquid soap formulation has multiple significance, but also a different effect on the physical and chemical characteristics at lower and higher concentrations. Functional and some physico-chemical characteristics of liquid hand washing soap depend on the pH value of the formulation. As the pH value increases, the surface tension and CMC increase to certain values, so pH=5.5 is taken as the optimal pH value of liquid soap, as a compromise between the values of the measured characteristics and the pH value of human skin. This research showed that the formulation containing 5.4 w/w% anionic surfactant, 1 w/w% amphoteric surfactant and 4 w/w% NaCl represents the optimal liquid soap formulation.

KEYWORDS: liquid soap; content of liquid soap; surfactants; electrolyte in liquid soap; characteristics of liquid soap; optimization of formulation

INTRODUCTION

To meet consumer expectations when buying liquid soap, it is necessary to take care of various characteristics such as washing power, pH value, density, visual appearance, and especially the viscosity of this product. Liquid soap should have an appropriate viscosity, enabling a smooth application process and ensuring even distribution and easier cleaning. Too low liquid soap viscosity values could lead to product leakage and give consumers the impression of poor cleaning. Excessively high viscosity of liquid soap affects difficult application and dispersion between hands during washing.

Liquid soaps are cleaning agents made of synthetic surfactants and auxiliary components, typically including anionic surfactants combined with amphoteric and nonionic surfactants. The type and relative concentration of the used surfactant determines the viscosity and other properties of the soap. In most cases, using a mix surfactants system leads to better interfacial properties, such as lower critical micelle concentration (CMC) and higher surface activity compared to single surfactants. This phenomenon is known as a synergistic effect [1], manifested by a significant increase in the viscosity of the solution [2]. Various additives, such as salts, can be added to liquid soap formulations to improve the properties of the final product. Some additives can enhance certain properties, while they can also have an adverse effect on other properties [3]. The addition of salt to an aqueous solution of surfactants changes the properties of the system, such as the critical micelle concentration, as well as the phase behavior of the surfactant solution [4].

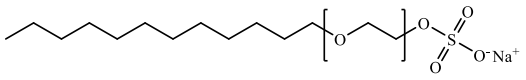
MATERIALS AND METHODS

The experimental part of the work refers to the preparation of 5 series (A, B, C, D and E series) of liquid soap formulations and the determination of the optimal composition of the prepared

formulations. The following components were used to prepare liquid soap formulations:

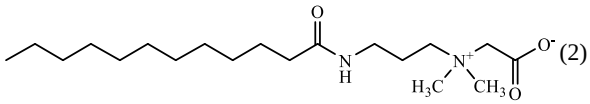
- sodium lauryl ether sulfate (Texapon N 70) – anionic surfactant (AS),
- cocamidopropyl betaine (Betadet HR) – amphoteric surfactant (AMS),
- sodium chloride (p.a.) and
- formaldehyde (36-38%, p.a.).

Sodium lauryl ether sulfate (Texapon N 70) is a highly concentrated anionic surfactant, a fatty acids alcohol derivative with 12-14 carbon atoms. It is a commercial product intended for the preparation of facial care and cleansing formulations, liquid soaps, shower gels, etc. Betadet HR is an amphoteric surfactant containing anionic (electronegative) and cationic (electropositive) polar groups. It is characterized by low toxicity, insensitivity to water hardness and compatibility with the skin. The basic roles of liquid detergents are thickening, foam reinforcement, emulsification, antiseptic action, and others.



(1)

Sodium lauryl ether sulfate



(2)

Cocamidopropyl betaine

Series A of liquid soaps was prepared as follows. A certain mass of anionic surfactant is weighed and dissolved in a glass beaker using a magnetic stirrer. A certain mass of amphoteric surfactant is measured in another glass beaker and mixed until homogenized. The prepared solutions are then mixed, and the pH value of the resulting solution is adjusted (with aqueous solution of NaOH or aqueous solution of HCl). The instrument used to measure the pH value of liquid soap formulations is a pH meter with a glass electrode (*Universal Meter, Multiline P4, WTW*). At the end, 2 drops of formaldehyde are added to the formulation to preserve it. The main goal of preparing this series of samples is to examine the influence of the pH value of liquid soap on the physico-chemical parameters and to determine the optimal pH value. Formulations of liquid soap series A are shown in Table 1.

Table 1. Formulations of liquid soap serie A

Sample	Texapon N 70 (w/w%)	Betadet HR (w/w%)	Formaldehyde	pH
A_1	5.4	1	2 drops	3
A_2	5.4	1	2 drops	5
A_3	5.4	1	2 drops	7
A_4	5.4	1	2 drops	9
A_5	5.4	1	2 drops	11

Series B of liquid soap formulations (Table 2) was prepared in a similar way as series A, with the fact that in these recipes, sodium chloride was present in different concentrations, which was added to the system after mixing the surfactant solution. All samples of serie B were adjusted to pH=5.5.

Table 2. Formulations of liquid soap serie B

Sample	Texapon N 70 (w/w%)	Betadet HR (w/w%)	NaCl (w/w%)	Formaldehyde	pH
B_1	5.4	1	1	2 drops	5.5
B_2	5.4	1	2	2 drops	5.5
B_3	5.4	1	3	2 drops	5.5
B_4	5.4	1	4	2 drops	5.5
B_5	5.4	1	5	2 drops	5.5
B_6	5.4	1	6	2 drops	5.5
B_7	5.4	1	7	2 drops	5.5
B_8	5.4	1	8	2 drops	5.5
B_9	5.4	1	9	2 drops	5.5
B_{10}	5.4	1	10	2 drops	5.5

Series of liquid soap formulations named C, D and E were prepared in the same way as series A and B. The pH value of these formulations was adjusted to pH=5.5 with the fact that these three formulations contain sodium chloride at a concentration of 4 w/w%. The main goal of preparing these groups of samples is to examine the effect of surfactant concentration on the characteristics of liquid soap formulations. Recipes of sample series from groups C, D and E are shown in Tables 3, 4 and 5.

Table 3. Formulations of liquid soap serie C

Sample	Texapon N 70 (w/w%)	Betadet HR (w/w%)	NaCl (w/w%)	Formaldehyde	pH
C_1	4.4	1	4	2 drops	5.5
C_2	6.4	1	4	2 drops	5.5

Table 4. Formulations of liquid soap serie D

Sample	Texapon N 70 (w/w%)	Betadet HR (w/w%)	NaCl (w/w%)	Formaldehyde	pH
D_1	5.4	0	4	2 drops	5.5
D_2	5.4	2	4	2 drops	5.5

Table 5. Formulations of liquid soap serie E

Sample	Texapon N 70 (w/w%)	Betadet HR (w/w%)	NaCl (w/w%)	Formaldehyde	pH
E_1	5.4	0	4	2 drops	5.5
E_2	0	5.4	4	2 drops	5.5

All samples of prepared liquid soap formulations were determined for physicochemical parameters, including density, viscosity, surface tension, and critical micelle concentration. Determining the density of liquid soap samples is based on measuring the mass *m* of a previously thermostated sample at 20 °C in a metal pycnometer with volume *V*. The density is calculated using the formula:

$$\rho = \frac{m}{V} \left\lfloor \frac{g}{cm^3} \right\rfloor \quad (3)$$

Cannon-Fensky viscometer was used to determine the viscosity of liquid soap samples. The determination of viscosity is based on measuring the time it takes for the thermostated liquid to efflux from the first to the second mark on the viscometer. The measurements were repeated 5 times, and the mean value of the liquid efflux time (t_{av}) was multiplied by the viscometer constant *C* to obtain the value for the kinematic viscosity of the tested sample, according to the formula:

$$\nu = C \times t_{av} \left[\frac{mm^2}{s} \right] \quad (4)$$

In this work, the stalagmometric method of determining the surface tension was applied. Surface tension was measured using a stalagmometer on samples prepared as solutions of liquid soaps with a concentration of 2 w/w%. After preparation, the samples were thermostated at a temperature of 20 °C. The lower part of the stalagmometer is immersed in the sample. The liquid is withdrawn above the upper mark on the stalagmometer. Counting drops starts when the liquid level coincides with the upper mark and ends when the liquid level coincides with the lower mark on the stalagmometer. The procedure is repeated several times, and the mean value is calculated.

The formula used to calculate the surface tension is:

$$\sigma_x = \sigma_{H_2O} \frac{\rho_x n_{H_2O}}{\rho_{H_2O} n_x} \left[\frac{mN}{m} \right] \quad (5)$$

where:

ρ_x - density of test liquid at the set temperature (kg/m^3),

ρ_{H_2O} - density of distilled water at the set temperature (kg/m^3),

n_{H_2O} - mean number of drops of distilled water and

n_x- mean number of drops of test liquid.

The critical micelle concentration (CMC) was determined from the graphical presentation of the measured values of electrical conductivity of liquid soap solutions of different concentrations, using a conductometer. For testing, 100 mL of liquid soap sample solution with a concentration of 1 w/w% was prepared, from which a series of dilutions of different concentrations was made. Electrical conductivity was measured from lower to higher sample concentrations at a temperature of 20 °C.

RESULTS AND DISCUSSION

The results of research are presented graphically using Microsoft Excel-prepared diagrams.

Samples of liquid soaps of series A show slight differences in the value of density and dynamic viscosity determined at a temperature of 20 ºC. The value of CMC is determined from the diagram of the dependence of electrical conductivity on the logarithm of the liquid soap concentration as a coordinate where two lines of different directions intersect. For both lines, the linear equations and the correlation factors are determined, which indicate their linearity. For sample A_1, the critical micelle concentration value is 0.203 w/w%. Sample A_1 was separated from the others as an example in order to explain the method of CMC determination (Figure 1). The following diagram (Figure 2) shows CMC values for the entire series of liquid soap samples of different pH values. This diagram shows that as the pH value increases, the CMC value increases until pH=5 (sample A_2). After pH=5, the CMC value starts to decrease again. The explanation for this can be found within the structure of the surfactants that make up the liquid soap formulation. Betadet is an amphoteric surfactant widely used in cosmetics and personal hygiene products, acting as a thickener, foam enhancer, and mildness enhancer [5]. This surfactant contains two polar groups, carboxyl (anionic) and quaternary ammonium group (cationic) [6], while anionic surfactant has one sulfated, negatively charged group. At higher pH values of liquid soap, the amphoteric surfactant behaves as an anionic compound because surfactants are deprotonated, and a negatively charged diffuse layer appears on their surface, which leads to the rejection of these substances. At lower pH values (acidic medium), the carboxyl group of the amphoteric surfactant is not ionized, but protonation of the tertiary

amine of the amphoteric surfactant occurs, whereby a positively charged diffuse layer appears on the surface of this surfactant, so the surfactant behaves as a cationic surfactant in these conditions [2]. In the case of anionic surfactant, there is no change in the surface charge. The change in the surface charge of the surfactant at lower pH values causes their attraction, which results in a shift in the concentration of CMC formation to lower values [7].

The head charge of an amphoteric surfactant, combined with anionic surfactant, plays an important role in forming mixed micelles [2]. It is stated in the literature that the pH value of Betadet at which the strength of positive and negative charges are equal (isoelectric point) is 6.25, so at pH=5, it behaves slightly cationic. Therefore, the slightly positively charged molecules of the amphoteric surfactant are placed between the negatively charged head groups of the anionic surfactant, which promotes a tighter packing of the monomers [8].

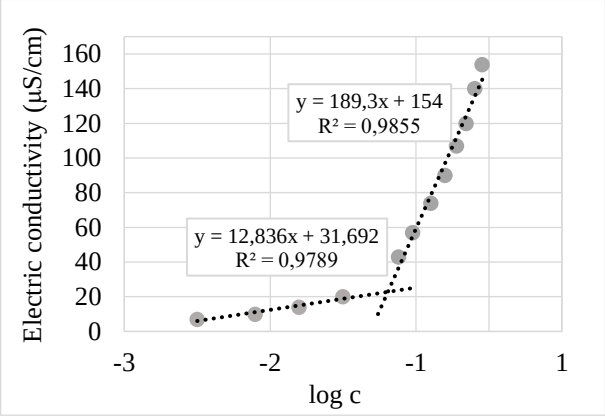


Figure 1. CMC of sample A_1

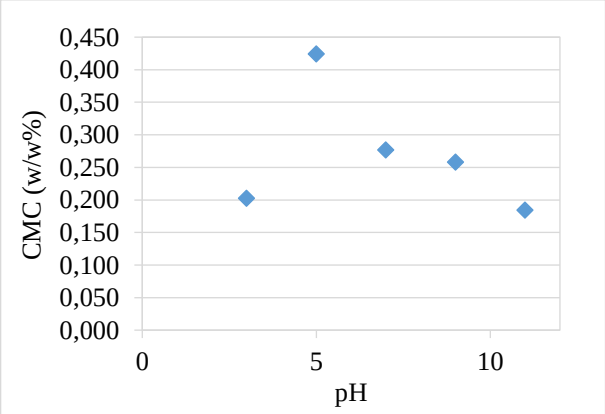


Figure 2. CMC of formulations serie A

Figure 3 shows that sample A_1 has the lowest value of surface tension, i.e., the sample whose pH value is set to pH=3. Also, it can be noticed that with an increase in the pH value, the surface tension increases to pH=7, after which it decreases slightly. In one study, it is stated that at low pH values, the CMC of sodium lauryl sulfate (anionic surfactant) decreases, and this decrease can occur either by replacing heavier ions with lighter ions or by reducing the charge on the surface of the micelle, thus changing the stability of the micelle [9]. A similar conclusion could be made for the SLES present in the samples of serie A.

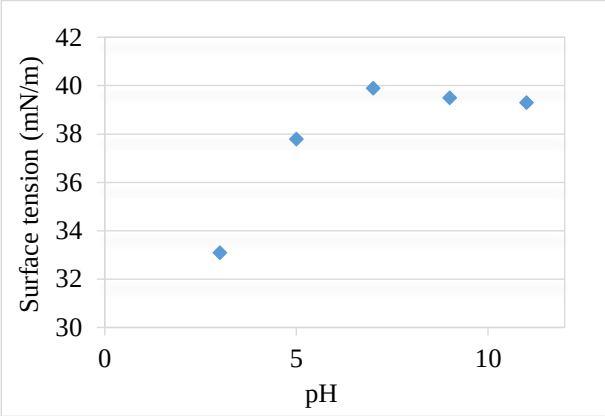


Figure 3. Dependence of surface tension on the pH value of the formulations of serie A

For the tested range of pH values, at pH=3, the liquid soap formulation has the lowest surface tension value. On the other hand, the pH value of the skin's balance is 5.5, so a soap with higher acidity would cause burning and irritation of sensitive skin. For this reason, pH=5.5 is taken as the optimal pH value of the liquid soap formulation. Therefore, all other samples were adjusted to that pH value.

Figures 4 and 5 show the dependence of density and viscosity on the concentration of sodium chloride present in the liquid soap formulation. It can be seen here that the density of liquid soap increases with the increase in sodium chloride concentration, and this growth is described by a linear function, which is also confirmed by the high value of the correlation coefficient R^2=0.9961. The change in viscosity with increasing NaCl concentration in the prepared formulations shows a characteristic shape similar to the curve of a Gaussian normal distribution. As stated in the literature, the change in viscosity is mainly a consequence of the transformation of their micellar morphology and structure [8]. The micellar structure changes from spherical to cylindrical, worm-shaped, and finally to branched micelles, which results in a non-monotonic viscosity trend in the form of the so-called "salt curve" [10]. Our research shows that the peak on the salt curve corresponds to the formulation containing 7 w/w% NaCl. Another study reported that formulations containing 7.5 w/w% sodium lauryl ether

sulfate and 2.5 w/w% cocamidopropyl betaine showed a viscosity peak at 2 w/w% NaCl, where measurements were made at 22 °C [8]. Comparing them, it can be concluded that the viscosity of these systems is affected by the concentration of surfactants, temperature, the presence of co-surfactant, but also other factors such as the molecular structure of the surfactant, polarity, presence of other additives [10]. Such solutions of surfactants in which worm-like micelles are formed are used as viscosity modifiers and regulators [2].

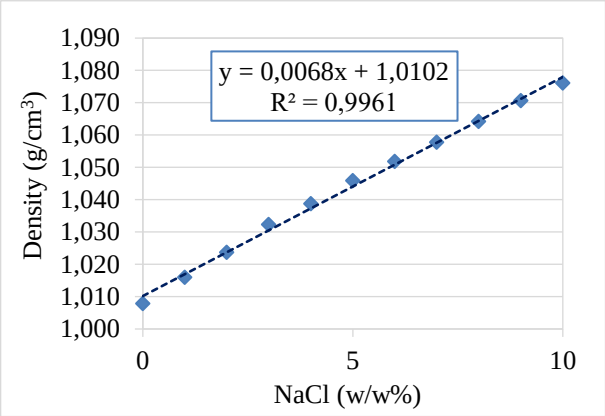


Figure 4. Density of formulations B serie

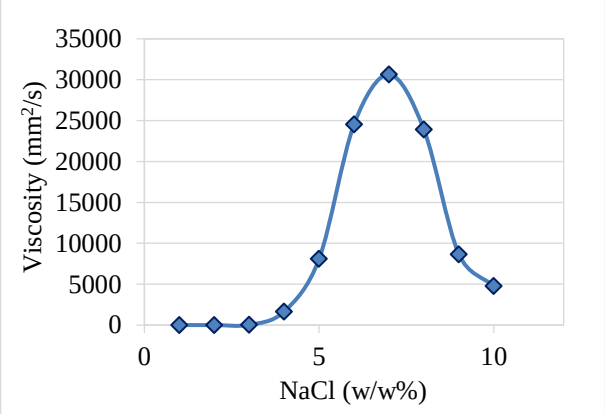


Figure 5. Viscosity of formulations B serie

After reaching the maximum on the salt curve, a further increase of NaCl concentration in the formulations leads to a decrease in viscosity due to the overlapping of worm-like micelles and the formation of a tangled network [11].

Figure 6 shows the dependence of the surface tension on the concentration of the liquid soap formulation of series B. The surface tension of solutions of liquid soaps decreases with increasing concentration of NaCl, so that at the highest concentration of NaCl it shows the lowest value of surface tension. The positive sodium cation and negative chloride anion of the electrolyte interact with the surfactant, reducing the electrostatic repulsion between the surfactant monomers in the solution. At the interface, there is more space for monomers that combine more compactly and further lower the surface tension [12]. On the other hand, when tested using a pressure piston pump, liquid soap formulations containing lower concentrations of sodium chloride showed a more uniform soap release, while the presence of higher concentrations of NaCl (5 w/w% and above) caused certain dosing disadvantages such as sliminess, stickiness, increased effort during extrusion, lumps, and the appearance of bubbles. For further tests, the formulation with 4 w/w% NaCl was selected as the formulation containing the optimum sodium chloride concentration because it performs better for user needs.

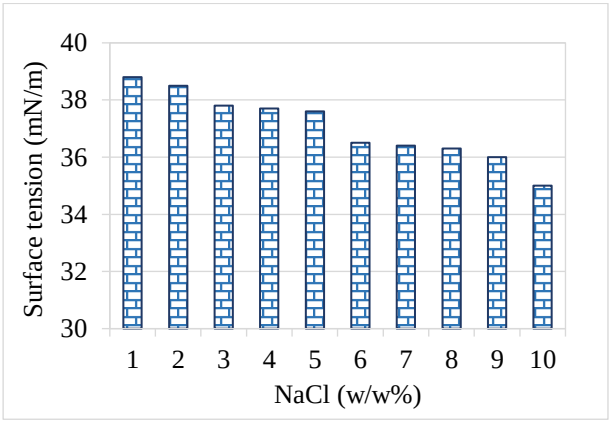


Figure 6. Surface tension of serie B

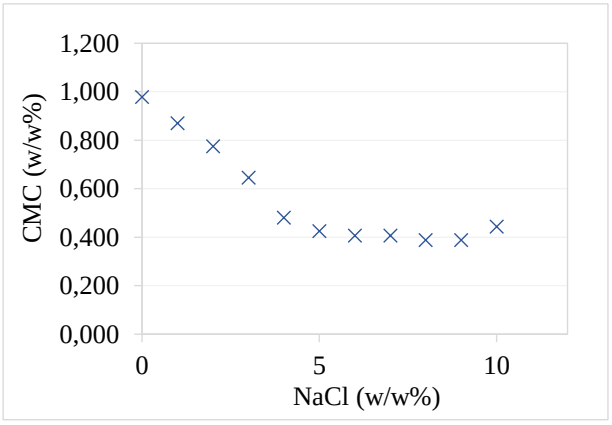


Figure 7. CMC of samples serie B

It is stated in the literature that the presence of electrolytes mainly causes a decrease in the critical micelle concentration, with this effect being most prominent with ionic surfactants. In contrast, with nonionic and amphoteric surfactants, this effect is smaller and often reversed [4]. This research showed an evident effect of the addition of NaCl on the reduction of the CMC of liquid soap made of a mixture

of anionic and amphoteric surfactants (Figure 7). The addition of sodium chloride leads to a decrease in the electrostatic repulsion between the charged surfactant groups inside the micelle, which allows a denser packing of the surfactant molecules and, thus, a decrease in the CMC [12]. The change in CMC is mainly attributed to the "salting in" or "salting out" of hydrophobic surfactant groups by the electrolyte present in the aqueous system [4], whose effect depends on the charge and ion radius of the electrolyte itself [12].

The dependencies of the density and viscosity of the liquid soap on the concentration of the anionic surfactant SLES are shown using diagrams in Figures 8 and 9. With the increase in the concentration of sodium lauryl ether sulfate (SLES), the density and viscosity of the liquid soap increases. Diagram 9 shows a greater change in viscosity when looking at samples C_1 (4.4 w/w% SLES) and C_2 (5.4 w/w% SLES), where a greater interaction of the anionic surfactant with the electrolyte is assumed, in contrast to the higher concentrations, if we look at the samples C_2 (5.4 w/w% SLES) and C_3 (6.4 w/w% SLES).

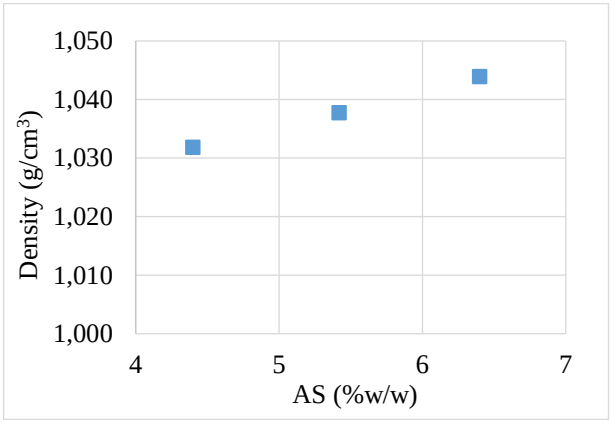


Figure 8. Density of samples serie C

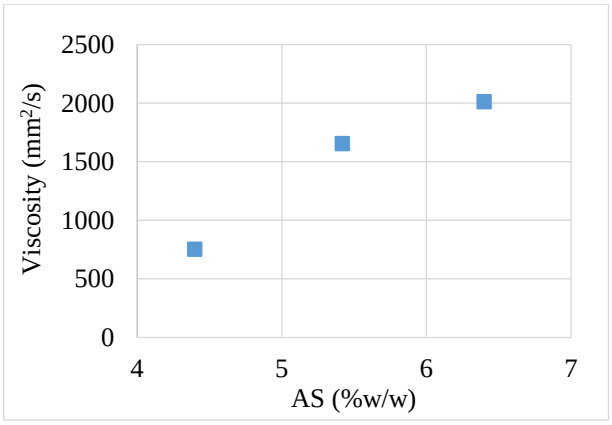


Figure 9. Viscosity of samples serie C

The surface tension measured for liquid soap formulations from series C shows very little change (Figure 10). The critical micelle concentration increases slightly with increasing SLES concentration (Figure 11). Of all tested samples of series C, sample C_3 shows the highest CMC value, which indicates that the present concentration of NaCl is not sufficient for maximum interaction with the increased concentration of anionic surfactant. In the work of Danov et al., 2004, formulations containing sodium dodecyl sulfate, alkylamidopropyl betaine and 10 mM NaCl (pH=5.5) were tested. The results of that research showed that with an increase in the concentration of anionic surfactant, the value of CMC increases, but the value of the surface tension is practically constant [13], as in this research.

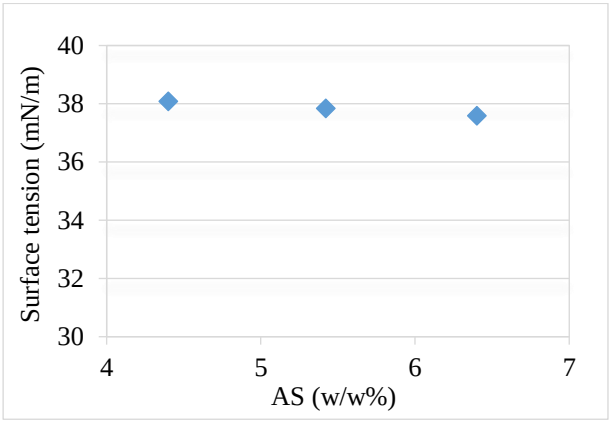


Figure 10. Surface tension of C serie

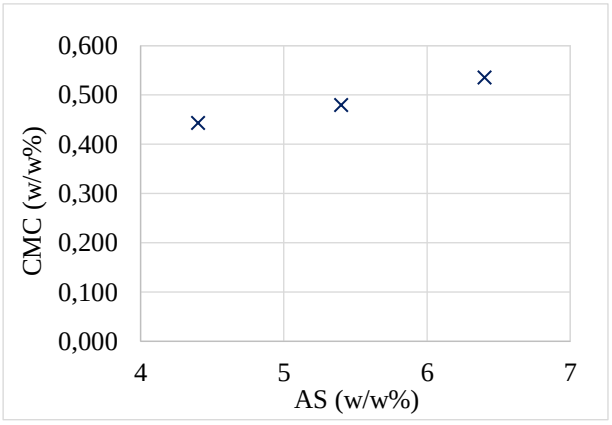


Figure 11. CMC of liquid soap C serie

Figures 12 and 13 shows that increasing the concentration of the amphoteric surfactant in the liquid soap formulation, density and viscosity increases. The synergistic effect of the mixture of anionic and amphoteric surfactants is particularly prominent in the case of an increase in the amphoteric surfactant concentration from 1 w/w% (1654 mm^2/s) to 2 w/w% (79 558 mm^2/s), which is a much higher

value than the maximum viscosity on the salt curve (Figure 5). In the research of the group of authors, it was stated that the sudden increase in viscosity of mixed solutions of surfactants is attributed to the transition of spherical micelles into rod-shaped ones, as well as an increase in the size of the micelles during the transition [14]. Literature data show that the concentration of NaCl required to create a viscosity peak decreases with an increase in the concentration of the amphoteric surfactant alkylamidopropyl betaine [8],[15], which could be investigated in the continuation of the current research.

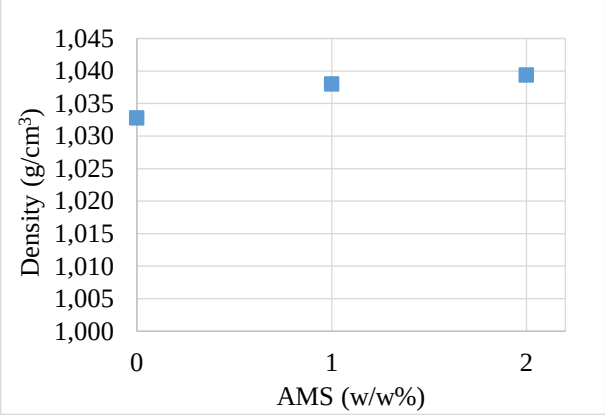


Figure 12. Density of samples serie D

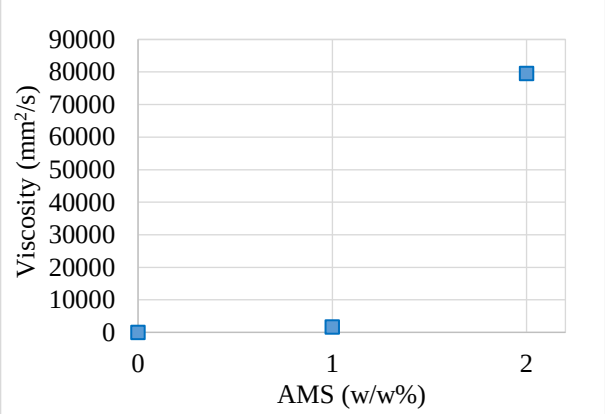


Figure 13. Viscosity of samples serie D

As Figure 14 shows, the surface tension of the solution decreases as the concentration of amphoteric surfactant in the formulation increases, while increasing the concentration of this amphoteric surfactant does not have a large effect on the reduction of CMC [12], as shown in Figure 15.

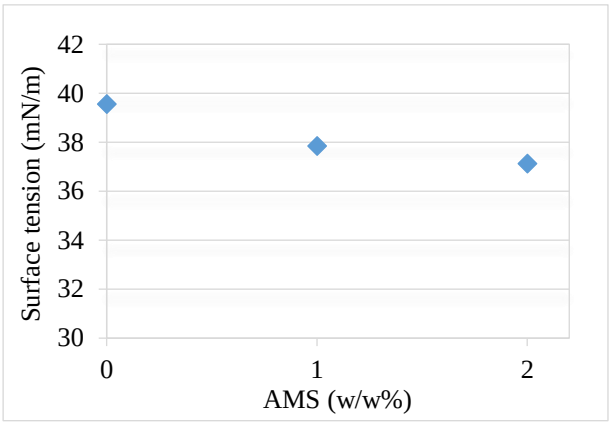


Figure 14. Surface tension of D serie

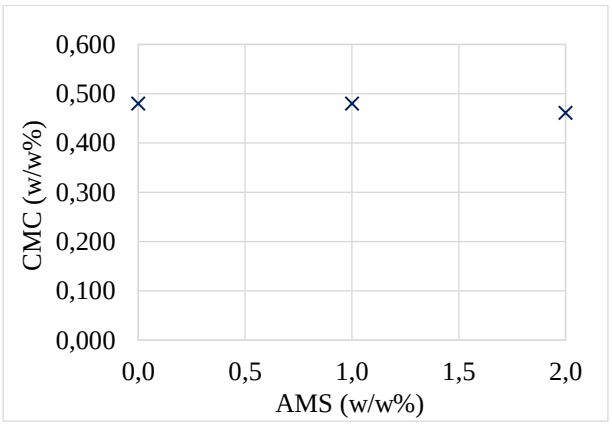


Figure 15. CMC of serie D

For the same concentration of one type of surfactant in the absence of another type of surfactant, it can be noted that the anionic surfactant contributes to a higher viscosity and density of liquid soap (Figure 16 and Figure 17) but also a higher surface tension and CMC than the used amphoteric surfactant (Figure 18 and Figure 19). Based on all the results of this test, it can be noted that mixed solutions of anionic and amphoteric surfactants in certain combinations show superior characteristics compared to solutions of individual surfactants, which coincides with the literature report [14].

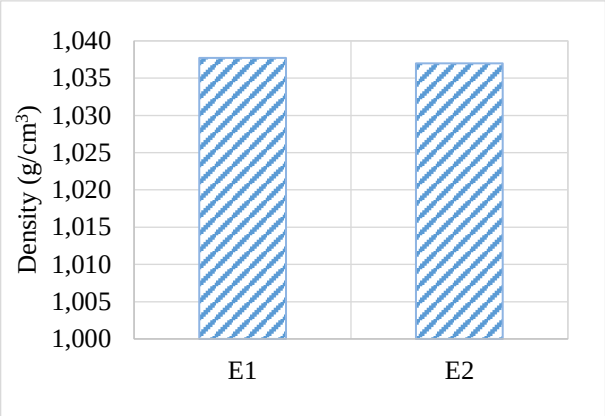


Figure 16. Density of samples serie E

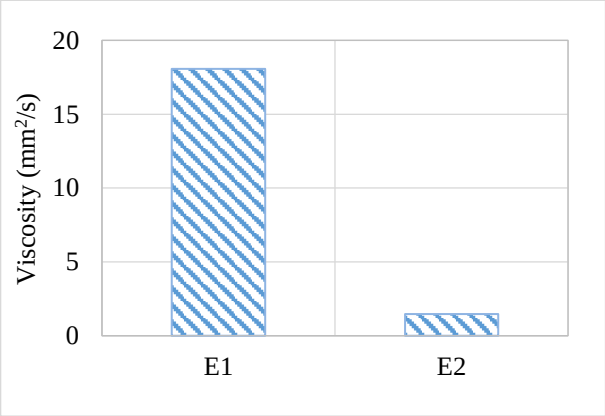


Figure 17. Viscosity of samples serie E

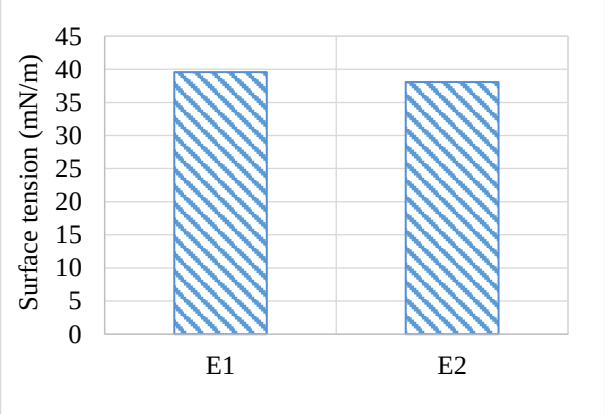


Figure 18. Surface tension of E serie

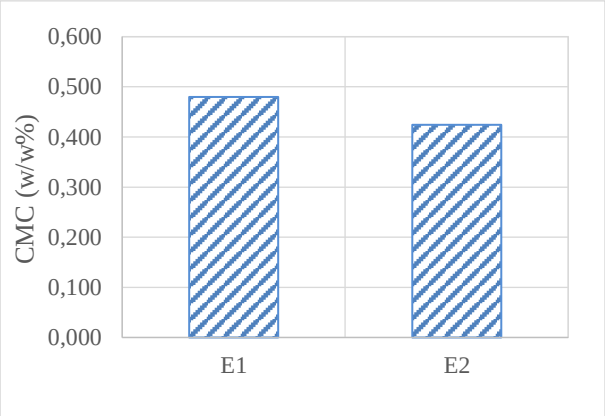


Figure 19. CMC of E serie

CONCLUSION

The functional and some physicochemical characteristics of liquid hand washing soap depend on several factors, including the pH value of the formulation. pH=5.5 is presented as the optimal pH value of liquid soap, as a compromise between the values of the measured characteristics and the pH value of human skin. As the concentrations of amphoteric and anionic surfactants in liquid soap increase, so do the density and viscosity values. The value of the surface tension decreases with the increase in the concentration of the present surfactants, with amphoteric surfactant making a greater contribution to the reduction. Regardless of certain advantages that the anionic surfactant shows in relation to the amphoteric one, the best characteristics of the liquid soap are shown by the formulation that contains a combination of both used surfactants. Adding NaCl to a liquid soap formulation has a different effect on the physico-chemical characteristics at lower and higher concentrations.

An increase in NaCl concentration leads to a change in the structure of the surfactant aggregates, which also changes the way the surfactant is packed in the micelles, which contributes to the transition of spherical micelles into rod-shaped micelles and then into wormlike micelles, which grow and overlap and create a transient interlaced network. These structural changes of the micelles are reflected in different effects on the physico-chemical characteristics, resulting in a liquid soap formulation containing 4 w/w% NaCl as the optimal concentration for an acceptable value of viscosity and stability of the formulation.

The desired properties of liquid soap require careful selection of the types and concentrations of appropriate surfactants and additives, where the formulation process still requires much time and research. In this research, the optimal formulation was

presented as containing 5.4 w/w% of anionic surfactant Texapon N 70 and 1 w/w% of amphoteric surfactant Betadet HR, in the presence of NaCl (4 w/w%), at pH=5.5. For the optimal liquid soap formulation, the values of physico-chemical parameters are: density 1.0387 g/cm^3, viscosity 1654 mm^2/s, surface tension 37.7 mN/m and CMC 0.48 %w/w measured at a temperature of 20 ºC.

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RESEARCH OF THE EFFICIENCY OF ORGANIC MATTER REMOVAL FROM WATER USING SYNTHETIC ZEOLITE

ORIGINAL SCIENTIFIC PAPER

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DOI: 10.51558/2232-7568.2023.17.1.59

RECEIVED 2024-06-05 **ACCEPTED** 2024-07-05

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ABSTRACT:
The paper investigated the effectiveness of using synthetic zeolite as an adsorbent for the removal of organic matter from water. Characterization of zeolite was performed using advanced testing methods: XRD, FTIR, BET and SEM/EDS. Raw water was analyzed for physicochemical characteristics before treatment (turbidity, color, pH, conductivity, content of organic matter, ammonia, nitrate and nitrite nitrogen, iron, manganese and chloride) and after treatment with an adsorbent (content of organic matter). Research on the efficiency of adsorption was carried out under the conditions of water temperature 25°C, individual doses of zeolite of 1, 2 and 4 g/L, mixing 200 rpm and adsorption time 60 minutes. The obtained results showed that by applying synthetic zeolite in a dose of 1 g/L, it is possible to achieve the removal of organic matter of 71.2%, while increasement of that dose up to four times has an insignificant effect on the adsorption efficiency.

KEYWORDS: synthetic zeolite; organic matter removal

INTRODUCTION

Water has a multiple and irreplaceable role in industry [1], [2], serving either as a basic or auxiliary raw material, or as an energy source, which is why ensuring sufficient quantities of water of appropriate quality is an industrial imperative. In supplying water for their needs, industrial facilities most often rely on surface sources such as rivers and lakes [3], [4]. However, the quality of the raw water of those sources is usually lower than that required for its specific purpose [3], [5], which is why it needs to be corrected.

One of the quality parameters of raw water, whose values are often inconsistent with those in industrial water quality requirements, is the content of organic matter, which is usually expressed by the permanganate index, i.e. consumption of $KMnO_4$ required for their oxidation [6]. Organic matter consists of a variety of particulate and dissolved carbon-based compounds [7] originating from natural sources such as soil runoff [8], aquatic life [9], microbial activity [10], as well as from anthropogenic sources including agricultural and urban runoff [11], [12], industrial discharges, effluents from sewage treatment plants [13] and septic systems [14]. Organic substances are commonly more present in surface than ground waters [15], [16], and urban natural water streams typically receive higher organic loads [17]. Water with a high content of organic matter is generally undesirable for industrial use due to its multiple negative impacts such as biofouling and biocorrosion in industrial systems [18] and inefficiencies in water treatment processes [19]. In addition, high levels of manganese are known to occur in organic-rich surface waters, sometimes as organically-complexed which is difficult to remove during conventional treatment [20].

In order to meet the requirements of standards and regulations on water quality for use in industry a number of methods are available for removing organic matter, such as: chemical coagulation [21], electrocoagulation [22], adsorption [23], advanced oxidation processes [24], membrane filtration [25] and biological treatment [26]. Among the listed, adsorption has advantages due to simple design and low investment costs and space requirements [27], which is why it is also used for other applications besides water treatment, such as: oil bleaching [28] and adsorption of volatile organic compounds from air [29].

Common adsorbents are: activated carbon [30], zeolites [31], silica gel [32] and bentonites [33]. Zeolites have high surface area and porosity that provide ample active sites for the adsorption of organic molecules [34] and can be modified in order to increase their adsorption capacity [35]. Although they are available in nature, they are more often synthesized, due to the simplicity of the procedure and higher purity of the product [36]. In this paper, the research of the effects of synthetic zeolite as an adsorbent in the removal of organic matter from natural surface water was carried out.

MATERIALS AND METHODS

In the experimental part of the research, the following materials were used: synthetic zeolite ZEOflair 110 (Zeochem, Zvornik), surface water of the Jala River sampled in the urban area of the city of Tuzla, and other reagents and chemicals required for zeolite characterization and physicochemical analysis of water.

The following methods were used to characterize the zeolite: X-ray diffractometry (XRD), infrared spectroscopy (FTIR), low-temperature nitrogen adsorption (BET) and scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDS). X-ray diffractometry was performed on a Rigaku Smartlab X-ray diffractometer, and crystalline phases were identified using Rigaku PDXL 2.0 software with the ICDD PDF-2 2016 database. Infrared spectroscopy was performed on a Shimadzu infrared spectrophotometer, IRAffinity 1S, using the ATR method (MIRacle 10). With this method, spectra were recorded in a wavenumber range of 4000 - 500 cm^{-1}. Low-temperature nitrogen adsorption was performed on the Micrometrics ASAP 2010 device, which determined the textural characteristics of the zeolite. Morphological characteristics were determined with an electron microscope JEOL-JSM-6460LV (Japan) at a resolution of 3-4 nm and 500-3000 times magnification. The samples were sputtered with gold on a BAL-TEC SCD 005 device, with a current of 30 mA, from a distance of 50 mm for 80 s. Microelement analysis was performed with an energy dispersive spectrometer with a Noran System Six 200 analyzer (detection of elements $Z \geq 5$, detection limit ~ 0.1% m/m, resolution 126 eV).

The physicochemical analysis of water before adsorption treatment included the determination of pH and electrical conductivity by potentiometric methods, content of organic matter, iron, manganese and chloride by volumetric methods, ammoniacal, nitrate and nitrite nitrogen by spectrophotometric methods. The content of organic matter in water was determined also after adsorption treatment.

The adsorption operation was carried out in laboratory conditions at room temperature, by adding a certain amount of zeolite (0.25, 0.5, 1.0 g) to a glass beaker with 500 mL of sampled water and simultaneously mixing the water with a magnetic stirrer at a speed of 200 revolutions per minute and a total mixing time of 60 minutes. After the specified time, the adsorbent was separated from the water by filtration on filter paper (blue strip), and then the content of organic matter was determined in the filtrate. The efficiency of removing organic matter from water (E_{om}) was calculated using the following relation:

$$E_{om}\ (\%) = (OM_{rw} - OM_{tw})/\ OM_{rw}$$

where OM_{rw} and OM_{tw} are contents of organic matter in raw and treated water.

RESULTS AND DISCUSSION

RESULTS OF CHARACTERIZATION OF SYNTHETIC ZEOLITE

Figure 1 shows the diffractogram of synthetic zeolite ZEOflair 110. Based on the obtained values of intensity I (imp) and interplane distances d (Å), and by comparison with literature data and ICDD standards, it was determined that the examined zeolite sample has a crystal structure of ZSM-5 (MFI) zeolite.

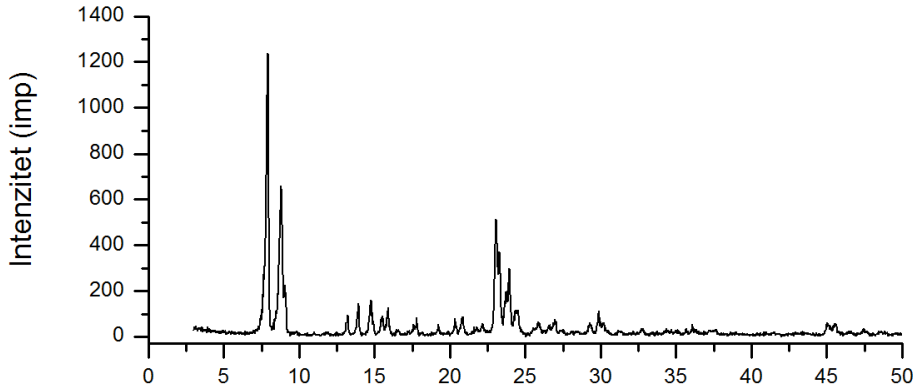

Figure 1. X-ray diffractogram of zeolite ZEOflair 110

The FTIR spectrum of zeolite ZEOflair 110 (Figure 2) shows bands that are characteristic of zeolite type ZSM-5. The bands at ~1225 cm^{-1} and ~1070 cm^{-1} originate from external and internal asymmetric stretching vibrations of Si-O-Si bonds, while the band at ~790 cm^{-1} originates from symmetrical stretching vibrations of Si-O-Si bonds.

The bands at ~590 cm^{-1} and ~545 cm^{-1} originate from double ring vibrations.

The adsorption isotherm of zeolite, shown in Figure 3 is of Type I, which is characteristic of microporous materials [37].

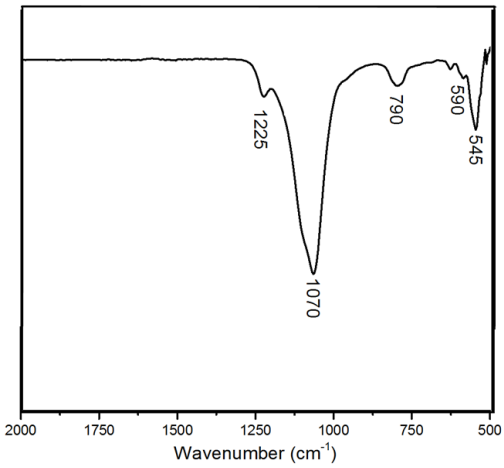

Figure 2. FTIR spectrum of zeolite ZEOflair 110

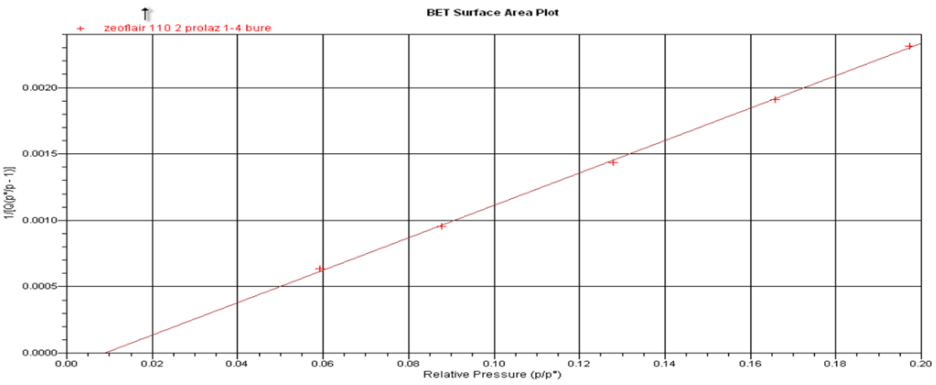

Figure 3. Adsorption isotherm of zeolite ZEOflair 110

The results of the analysis of textural characteristics of zeolites (table 1) show that the specific surface is 336.88 m^2/g, which is within the typical range (300-2000 m^2/g) of their specific surfaces measured by gas adsorption [38]. From the given data, it follows that the share of micropore surface area in zeolite amounts to about 71.65% of the total specific surface area determined by the BET method.

Table 1. Textural characteristics of zeolite ZEOflair 110

Characteristics	Value
Specific surface area (SP_{BET}), m^2/g	336.88
Constant, C_{BET}	-71.34
External specific surface area (SP_{ext}), m^2/g	95.49
Micropore surface area ($S_{\mu p}$), m^2/g	241.39
Micropore volume ($V_{\mu p}$), cm^3/g	0.1208
Mean pore diameter (d_p), nm	3.287

SEM micrographs of zeolite at magnifications of 500, 1000 and 3000 times are given in Figure 4, where it can be seen that the examined material has a regular spherical crystal grain shape.

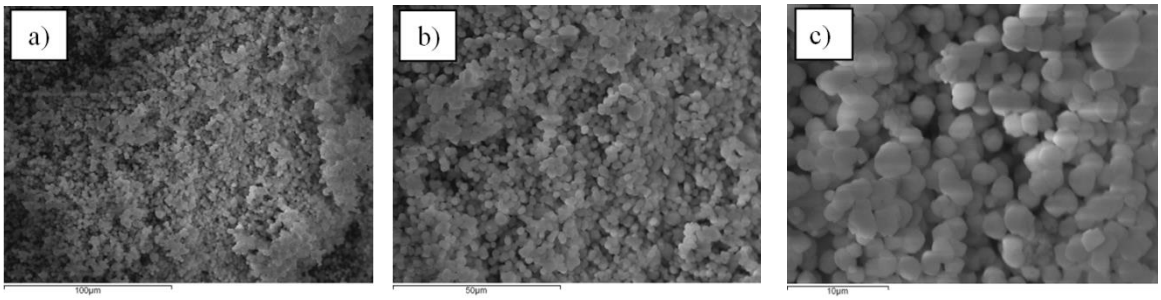

Figure 4. SEM micrographs of synthetic zeolite ZEOflair 110 at magnifications: a) 500x, b) 1000x and c) 3000x

The SEM analysis was done in combination with X-ray energy dispersion (EDS) analysis (Fig. 4) revealing the elemental composition (wt%) of the zeolite sample, which represents silicalite: 59.10% O, 40.09% Si, 0.81% Na. Based on EDS analysis of the zeolite, high aluminosilicate module (SiO_2/Al_2O_3 > 500) was calculated.

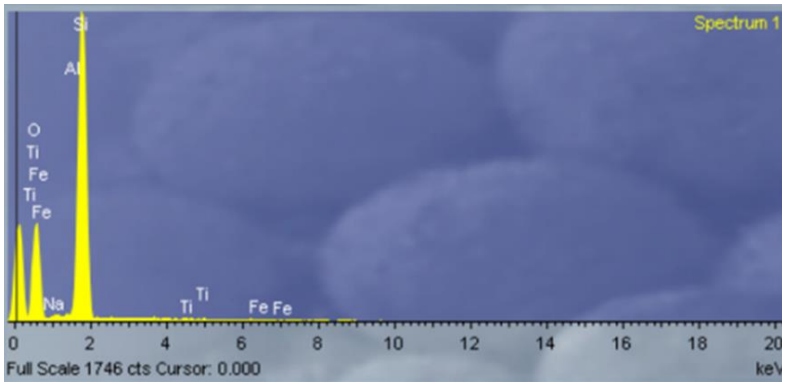

Figure 5. EDS spectrum of zeolite ZEOflair 110

RESULTS OF WATER ANALYSIS

The results of the physicochemical characteristics of the untreated water sample, shown in Table 2, are mostly within the range that is characteristic for urban surface watercourses. The electrical conductivity of rivers generally ranges from 50 to 1500 μS/cm [39], and pH from 6.5 to 8.5 [40]. The Jala River has a high content of organic matter (table 3), which is expected considering the urban environment through which it flows and the exposure of the water to the surrounding environment, therefore the origin of organic matter can be either from products of plant and animal life, or communal and industrial discharges. The high chloride content in the surface water of the Jala River in Tuzla, Bosnia and Herzegovina, probably originates from the underground salt deposits for which this geographic area is known, which can significantly affect chloride levels in local watercourses.

Table 2. Results of physicochemical characteristics of raw water

Parameter	Value
pH	7.49
Electrical conductivity (μS/cm)	453
Organic matter as $KMnO_4$ consumption (mg/L)	113
Ammoniacal nitrogen (mg/L)	2.26
Nitrate nitrogen (mg/L)	0.47
Nitrite nitrogen (mg/L)	0.098
Iron (mg/L)	0.00
Manganese (mg/L)	0.021
Chlorides (mg/L)	40.00

RESULTS OF ORGANIC MATTER REMOVAL EFFICIENCY

Table 3 shows the results of adsorption of organic matter from water by zeolite ZEOflair 110, depending on the adsorbent dose.

Table 3. Efficiency of adsorption of organic matter by zeolite ZEOflair 110

Dose of adsorbent (g/L)	Consumption of $KMnO_4$ (mL)	Content of organic matter in treated water (mg/L)	Organic matter removal efficiency (%)
1	10.3	32.55	71.2
2	9.7	30.66	72.8
4	10.9	34.45	69.5

Increasing the dose of zeolite generally increases the efficiency of adsorption of organic matter from water. This is because more zeolite provides more surface area and more adsorption sites for organic molecules to adhere to. However, once the optimal dose is reached, further increases may result in only minor improvements. Adsorption processes eventually reach an equilibrium in which the rate of adsorption is equal to the rate of desorption. The results in Table 3 show that by increasing the dose of synthetic zeolite, the efficiency of adsorption of organic matter in water increased to a certain value, after which it decreased. This phenomenon is generally due to several factors. First, higher doses of zeolite can lead to particle aggregation, which reduces the effective surface area available for adsorption. Aggregated particles have fewer available active sites for the adsorption process. Second, excessive amounts of zeolite can interfere with the mass transfer of organic molecules to the adsorption sites. This can create a situation where the diffusion of organic matter to the adsorbent surface is hindered, thus lowering the overall adsorption efficiency. Third, at higher doses, most of the easily accessible adsorption sites are quickly saturated. Additional zeolite does not significantly contribute to further adsorption because the remaining sites are less accessible or the concentration of organic matter in the solution is too low for effective adsorption.

A study investigating the removal of humic acid using surfactant-modified zeolite [41] indicated that after reaching an optimal zeolite dose, further increases did not improve and could even reduce adsorption efficiency due to these reasons. Another study on the adsorption of Congo red dye [42] also observed that beyond an optimal dose, the adsorption capacity did not increase and could decrease due to similar factors like particle aggregation and reduced mass transfer efficiency.

CONCLUSION

In this paper, the possibility of using the commercial synthetic zeolite ZEOflair 110 for the removal of organic matter from water was investigated. Characterization of zeolite was performed using advanced methods: X-ray powder diffractometry, infrared spectroscopy, low-temperature nitrogen adsorption and scanning electron microscopy with energy dispersive spectrometry. Based on the test results, it was determined that the examined zeolite sample has a crystal structure of ZSM-5 zeolite, and that it is a microporous material characterized by a type I adsorption isotherm. The zeolite has a regular spherical shape and is silicalite, in which the presence of aluminum was not detected, and it has a high modulus of SiO_2/Al_2O_3 (>500).

Synthetic zeolite ZEOflair 110 is an effective adsorbent in the treatment of water with a high content of organic matter; in this research, the dose of adsorbent of 1 g/L enabled the reduction of organic matter from the initial concentration of 113 mg/L to 32.55 mg/L, while with a double dose of adsorbent (2 g/L), the treatment efficiency was only slightly increased (1.5%). However, increasing the dose of synthetic zeolite beyond an optimal point can lead to a decrease in adsorption yield for removing organic matter from water, due to particle aggregation, interference with mass transfer and saturation of adsorption sites. Thus, it is crucial to determine the optimal dose of synthetic zeolite for a given water treatment application to ensure maximum efficiency without unnecessary material usage or adverse effects on the adsorption process.

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INFLUENCE OF MACERATION, SOLVENT TYPES, AND EXTRACTION DURATIONS ON THE YIELD OF MILK THISTLE SEEDS *(SILYBUM MARIANUM)* EXTRACTION

ORIGINAL SCIENTIFIC PAPER

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DOI: 10.51558/2232-7568.2023.17.1.67

RECEIVED 2024-06-09 **ACCEPTED** 2024-06-25

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ABSTRACT:
The study investigated the extraction yield of defatted Silybum marianum seed samples using maceration as the sole extraction technique. Different solvent types (methanol, ethanol, and water) and extraction durations were tested. Prior to extraction, the samples were ground and defatted with n-hexane. For each combination of solvent type, and extraction duration, the extracted mass (g of extract/g of defatted sample) was determined. The impact of each parameter on the yield was analyzed, revealing significant effects. Results showed that water-based maceration for 4 hours yielded the highest average mass of dry extract, followed by shorter durations at 2 hours. Ethanol occasionally outperformed methanol, particularly at the 2-hour mark, but methanol consistently produced lower yields across longer extraction durations. These findings emphasize the need for careful optimization of solvent type and extraction duration to maximize extraction yield. Subsequent analysis using Tukey's HSD test revealed significant differences in dry extract mass among solvents. Water yielded the highest at 2 and 4 hours, ethanol at 4 hours, and methanol at 4 hours as well.

KEYWORDS: Silybum marianum; maceration; solvent types; plant extraction, yield analysis

INTRODUCTION

Classical techniques for solvent extraction of plant compound matrices usually involve choosing a solvent and utilizing agitation, and/or heat. Traditional methods like maceration, Soxhlet extraction, and percolation are common but come with drawbacks such as being time-consuming, requiring large quantities of solvent, and potentially causing degradation of active compounds [1]. The selection of extraction methods varies based on factors such as the plant species or botanical characteristics [2]. For example, maceration is a cost-effective method where plant material is immersed in solvent for extraction [3].

Additionally, different solvents are employed to extract compounds from plants, with extraction outcomes influenced by solvent types [4; 5]. Achieving complete extraction without chemical alteration is essential [6]. Commonly used solvents include water and aqueous blends of ethanol, methanol, and acetone [7]. Studies indicate that aqueous ethanol is more effective than methanol and acetone, while, conversely, water surpasses 80% methanol or 70% ethanol in extracting certain compounds from tea [8;9].

In this study, the extraction dynamics of milk thistle seeds *(Silybum marianum)* seed samples was meticulously analyzed. Focus was placed on conventional maceration using methanol, ethanol, and water as solvents. With varying extraction durations, the aim was to optimize yield and quality. Preceding extraction, samples were ground and defatted with n-hexane. The goal was to discern the optimal combination of parameters for maximum extraction efficiency and quality..

MATERIALS AND METHODS

Milk thistle seeds (*Silybum marianum*) were ground into a fine powder with a particle diameter of 0.4 mm using a blender. The extraction process was conducted in two steps, starting with defatting. The ground sample was weighed and extracted with n-

hexane for 4 hours at room temperature, using a sample-to-solvent ratio of 20 grams of sample to 200 ml of petroleum ether. The samples were then filtered and dried to remove any remaining petroleum ether. For maceration extraction, 10 grams of defatted sample it was weighed into glasses and solvent was added. The samples were extracted with 200 ml of solvent for 2, 4, 6 and 8 hours. The solvents used were ethanol and methanol. After extraction, the solvent was removed using a rotary evaporator (BUCHI Rotavapor R-215) under the following conditions: bath temperature of 60°C, boiling point of 40°C and cooling water temperature of 20°C. The extract was then dried at 40°C to constant weight, and the extraction yield was calculated gravimetrically.

RESULTS AND DISCUSSION

After conducting the measurements, the yield results obtained are presented in Table 1. The highest average yield of milk thistle seeds (*Silybum marianum*) for extraction via maceration in five repetitions using water as a solvent was measured with an exposure time of 4 hours. The yield value was Me=1.0468 grams (g), with a standard deviation of SD=0.0045 g and a standard error of SE=0.0020 g. The 95% confidence interval (CI) for the mean ranged from 1.0411 g to 1.0524 g, with a value range from a minimum of 1.0392 g to a maximum of 1.0511 g. The lowest average yield for this solvent was recorded with an 8-hour exposure, yielding Me=0.510340 g, with SD=0.0182 g, an SE of 0.0081 g, a 95% CI from 0.4876 g to 0.5330 g, and a value range from a minimum of 0.5008 g to a maximum of 0.5430 g (Table 1).

For the ethanol (EtOH) solvent, the highest average yield was achieved with a 4-hour exposure, amounting to Me=0.687200 g with SD=0.0060 g and SE=0.0026 g. The 95% CI for the mean ranged from 0.6797 g to 0.6946 g, with a measured value range in five repetitions from a minimum of 0.6769 g to a maximum of 0.6916 g. The lowest average yield of dry milk thistle seed extract (*Silybum marianum*) using EtOH as a solvent was measured with an 8-hour exposure. The average yield was Me=0.397000 g, with SD=0.0327 g and SE=0.0146 g, while the 95% CI ranged from 0.3563 g to 0.4376 g, with a yield range from a minimum of 0.3635 g to a maximum of 0.4492 g (Table 1).

For maceration extraction using methanol (MtOH) as a solvent, the highest average yield in five repetitions was achieved with a 4-hour exposure. The average yield value was Me=0.8360 g with SD=0.0029 g and SE=0.0013 g, while the 95% CI ranged from 0.8322 g to 0.8397 g, with a value range from a minimum of 0.8312 g to a maximum of 0.8394 g (Table 1). The lowest average yield in five repetitions for this case was measured with a 2-hour extraction, yielding Me=0.4274 g with SD=0.0045 g and SE=0.0020 g, while the 95% CI for the mean was from 0.4217 g to 0.4330 g, with a value range from a minimum of 0.4198 g to a maximum of 0.4308 g (Table 1).

Table 1. Structure of milk thistle seed yields (*Silybum marianum*) during maceration extraction in water, EtOH, and MeOH at exposure times of 2, 4, 6, and 8 hours.

S	ET h	N	Me	SD	SE	95% Confidence Interval for Mean		Min.	Max.
						LB	UB		
Water	**2**	5	1.0351	0.0007	0.0003	1.0342	1.0359	1.0344	1.0363
	4	5	1.0468	0.0045	0.0020	1.0411	1.0524	1.0392	1.0511
	6	5	0.9526	0.0136	0.0060	0.9356	0.9695	0.9382	0.9702
	8	5	0.5103	0.0183	0.0081	0.4876	0.5330	0.5008	0.5430
	T	20	0.8862	0.2259	0.0505	0.7804	0.9919	0.5008	1.0511
EtOH	**2**	5	0.6376	0.0023	0.0010	0.6347	0.6405	0.6342	0.6403
	4	5	0.6872	0.0060	0.0026	0.6797	0.6946	0.6769	0.6916
	6	5	0.6467	0.0113	0.0050	0.6326	0.6607	0.6311	0.6621
	8	5	0.3970	0.0327	0.0146	0.3563	0.4376	0.3635	0.4492
	T	20	0.5921	0.1182	0.0264	0.5367	0.6475	0.3635	0.6916
MeOH	**2**	5	0.4274	0.0045	0.0020	0.4217	0.4330	0.4198	0.4308
	4	5	0.8360	0.0029	0.0013	0.8322	0.8397	0.8312	0.8394
	6	5	0.7421	0.0084	0.0037	0.7316	0.7526	0.7317	0.7539
	8	5	0.5490	0.0145	0.0065	0.5309	0.5671	0.5342	0.5650
	T	20	0.6386	0.1642	0.0367	0.5617	0.7155	0.4198	0.8394

Legend: S = solvent, ET = extraction time, h = hours, T = Total, Mean = (Me), Std. Deviation = (SD), Std. Error = (SE), LB = Lower Bound, UB = Upper Bound, Minimum = Min., Maximum = Max.

To evaluate the influence of solvent type and extraction time on the yield of dry mass of milk thistle (*Silybum marianum*) seed extract as the dependent variable, a two-way analysis of variance (ANOVA) of different groups was applied to examine the individual and combined effects of extraction time and solvent type as independent variables on the mean values of dry mass extract as the dependent variable. Preliminary analysis examined the conditions for the application of two-way ANOVA. The assumption of normal distribution was not significantly violated, and Levene's Test of Equality of Error Variances showed statistical significance: F (11, 48) =2.934, Sig.=0.005, indicating a violation of the assumption of variance homogeneity, thus requiring a stricter significance level of p = 0.05. A significance level of p = 0.01 is used for further analysis. The impact of solvent selection and extraction time on yield value was examined using two-way ANOVA of different groups, with extraction time in four time intervals: 2, 4, 6, and 8 hours (Table 2).

During extraction, a statistically significant main effect of solvent selection on the average value of dry extract mass was found: F (2, 48) = 2860.18, p = 0.000, with an effect size indicator of eta squared of 0.992, characterized as a strong effect. The main effect of extraction time on the average value of dry extract mass also proved to be statistically significant at the significance level of p = 0.01; F(3, 48) = 2201.28, Sig = 0.000, with an effect size that can be classified as large, partial eta squared is 0.993 (Table 2).

Table 2. Results of the application of two-way analysis of variance of different groups.

Source	SS	DF	MS	F	Sig.	Partial Eta Squared
CM	2.740[a]	11	0.249	1425.71	0.000	0.997
I	29.87	1	29.878	171004.60	0.000	1.000
OT	0.999	**2**	0.500	**2860.18**	**0.000**	**0.992**
VR	1.154	**3**	0.385	**2201.28**	**0.000**	**0.993**
OT * VR	0.587	**6**	0.098	**559.77**	**0.000**	**0.986**
Error	0.008	**48**	0.000			
a). R Squared = 0.997 (Adjusted R Squared =0 .996)						

Legend: SS = Sum of Squares CM = Corrected Model, I = Intercept, DF = degrees of freedom, MS = Mean Square, F-test, Sig. = p-value, Partial Eta Squared = Partial eta squared, Adjusted R Squared = Adjusted R squared.

The interaction of solvent type and extraction time was statistically significant; F (6, 48) = 559.77, p = 0.000. (Table 2) As the statistical significance of the interaction between solvent type, extraction time, and the average value of dry extract mass was determined, further exploration through subsequent tests is warranted. Therefore, we further conducted an analysis of simple effects by dividing the sample into groups and considering the dependence of the extracted mass of dry extract on the type of solvent used in:

- Group **g1**-when extraction was performed after 2 hours;
- Group **g2**-when extraction was performed after 4 hours;
- Group **g3**-when extraction was performed after 6 hours;
- Group **g4**-when extraction was performed after 8 hours.

[1]Cohen, J. W. (1988). Statistical power analysis for the behavioral sciences (2nd edn). Hillsdale, N: Lawrence Erlbaum Associates. According to Cohen's criteria, if the partial eta squared (r) is: (r = 0.1), the effect is small; (r = 0.3), the effect is medium; (r = 0.5) or higher, the effect is large.

The conditions for the application of one-way analysis were not violated, so a statistically significant difference was found, at a significance level of p=0.01, in the average value of the mass of extracted dry extract between solvents (water, EtOH, and MeOH). Subsequent comparisons in group g1 - extraction by maceration after 2 hours using the Tukey's HSD test showed that the actual differences in average mass of extracted dry extract, extracted after 2 hours, when water was used as the solvent (Me=1.0351, SD=0.0007), significantly differed from the average mass of dry extract when EtOH was the extraction solvent (Me=0.6376 g, SD=0.0023 g), with a mean difference, R=0.3974 g, Sig=0.000, as well as when MeOH was the solvent (Me=0.4274 g, SD=0.0045 g) with a mean difference R=0.6077 g, Sig=0.000, at a significance level of p = 0.01. (Table 3, Table 4, Figure 2).

Furthermore, the Tukey's HSD test showed that the average value of dry extract mass obtained using ethanol (Me=0.6376 g, SD=0.0023 g) significantly differed from the average mass of extracted dry extract

when methanol was used as the solvent, (Me=0.4274 g, SD=0.0045 g), with a mean difference R=0.2102 g, Sig. = 0.000, at a significance level of p = 0.01 in extraction conducted by maceration (Table 3, Table 4, Figure 1).

Table 3. Descriptive statistics of extracted dry extract mass after 2 hours of maceration extraction.

S	N	Me	SD	SE	95% CI Interval for Mean		Min.	Max.
					LB	UB		
Water	5	1.0351	0.0007	0.0003	1.0342	1.0359	1.0344	1.0363
EtOH	5	0.6376	0.0023	0.0010	0.6347	0.6405	0.6342	0.6403
MeOH	5	0.4274	0.0045	0.0020	0.4217	0.4330	0.4198	0.4308
Total	15	0.7000	0.2608	0.0673	0.5556	0.8445	0.4198	1.0363
ANOVA				**F(2,14)=54255,24,**			**Sig.=0.000**	

Legend: S = Solvent, Me = Mean, SD = Standard Deviation, SE = Standard Error, 95% Confidence Interval for Mean = 95% CI, Min = Minimum, Max = Maximum

Table 4. Results of post-hoc comparison of average mass of dry extract using Tukey's HSD test for actual differences in extraction after 2 hours.

(I) S	(J) S	MD (I-J)	SE	Sig.	99% Confidence Interval	
					LB	UB
Water	EtOH	0.3974*	0.0018	0.000	0.3907	0.4041
	MeOH	0.6077*	0.0018	0.000	0.6010	0.6143
EtOH	Water	-0.3974*	0.0018	0.000	-0.4041	-0.3907
	MeOH	0.2102*	0.0018	0.000	0.2035	0.2169
MeOH	Water	-0.6077*	0.0018	0.000	-0.6143	-0.6010
	EtOH	-0.2102*	0.0018	0.000	-0.2169	-0.2035
***. The mean difference is significant at the p=0.01 level.**						

Legend: S = solvent, MD = Mean Difference, Average Difference, SE = Standard Error, Sig. = Significance, 99% CI = 99% Confidence Interval, 99% Confidence Interval for Mean Difference.

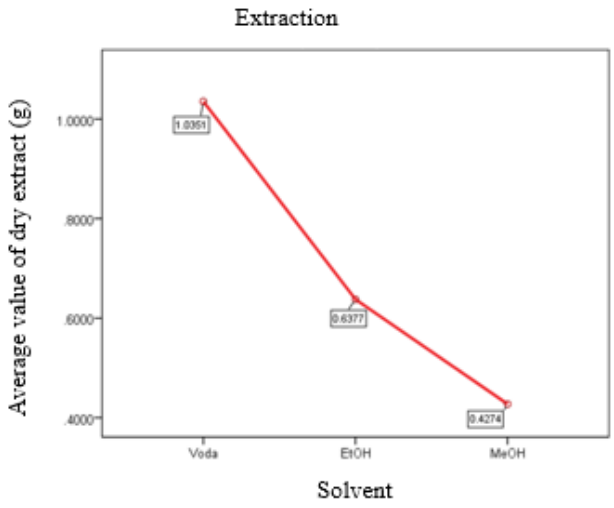

Fig. 1. Average values of extracted dry mass obtained through maceration extraction relative to the type of extraction solvent implemented after 2 hours.

Subsequent comparison within group g2 - maceration extraction after 4 hours using Tukey's HSD test for real differences showed that the average value of extracted dry mass when water was used as the extraction solvent (Mean=1.0468, SD=0.0045) significantly differs from the average dry mass value when ethanol (EtOH) was used as the solvent; (Mean=0.6872 g, SD=0.0060), with an average difference, R=0.3596 g, Sig.=0.000 and when methanol (MeOH) was the solvent; (Mean= 0.8360 g, SD=0.0029) with an average difference R= 0.2108 g, Sig=0.000, at a significance level of p = 0.01. (Table 5, Table 6, Figure 3).

Furthermore, subsequent comparison of the average values of extracted dry mass obtained using ethanol (Mean= 0.6872 g, SD=0.0060) significantly differs from the average value of extracted dry mass obtained using methanol as the extraction solvent (Mean= 0.8360 g, SD=0.0029) with an average difference R=-0.1488 g, Sig.=0.000, at a significance level of p = 0.01 in the maceration extraction method (Table 5, Table 6, Figure 2).

Table 5. Descriptive indicators of extracted dry mass of extract after 4 hours of maceration extraction.

S	N	Me	SD	SE	95% CI Interval for Mean		Min.	Max.
					LB	UB		
Water	5	1.0468	0.0045	0.0020	1.0411	1.0524	1.0392	1.0511
EtOH	5	0.6872	0.0060	0.0026	0.6797	0.6946	0.6769	0.6916
MeOH	5	0.8360	0.0029	0.0013	0.8322	0.8397	0.8312	0.8394
Total	15	0.8566	0.1527	0.0394	0.7720	0.9412	0.6769	1.0511
ANOVA				**F(2,14)= 7441.507,**		**Sig.=0.000**		

Legend: S = solvent, Me - Mean, SD - Standard Deviation, SE - Standard Error, 95% CI = 95% Confidence Interval for Mean, Min. = Minimum, Max. = Maximum

Table 6. Results of subsequent comparison of the average dry mass of extract using Tukey's HSD test for real differences in maceration extraction after 4 hours.

(I) S	(J) S	MD (I-J)	SE	Sig.	99% CI Confidence Interval	
					LB	UB
Water	EtOH	0.3596*	0.0029	0.000	0.3490	0.3701
	MeOH	0.2108*	0.0029	0.000	0.2002	0.2213
EtOH	Water	-0.3596*	0.0029	0.000	-0.3701	-0.3490
	MeOH	-0.1488*	0.0029	0.000	-0.1593	-0.1382
MeOH	Water	-0.2108*	0.0029	0.000	-0.2213	-0.2002
	EtOH	0.1488*	0.0029	0.000	0.1382	0.1593
***. The mean difference is significant at the 0.01 level.**						

Legend: S = solvent, MD = Mean Difference, Average difference SE = Std. Error, Standard Error Sig. = Significance 99% CI = 99% Confidence Interval.

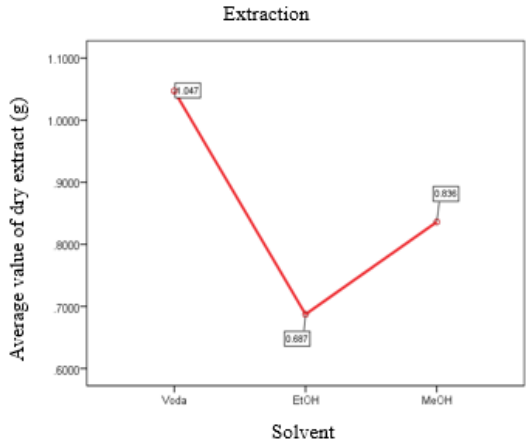

Fig. 2. Average values of extracted dry mass through maceration extraction relative to the type of extraction solvent implemented after 4 hours.

Subsequent comparison within group g3 - maceration extraction after 6 hours using Tukey's HSD test for real differences in the average values of extracted dry mass showed that the average dry mass value extracted by soaking in water (Mean=0.9526, SD=0) significantly differs from the average dry mass value extracted by ethanol (Me=0.6467, SD=0.0113), with an average difference, R=0.3059 g, Sig.=0.000, as well as from the average dry mass value extracted by methanol (Me= 0.7421, SD=0.0084) with an average difference R= 0.2104 g, Sig=0.000, at a significance level of p=0.01. (Table 7, Table 8, Figure 4). Furthermore, subsequent comparison of the average values of extracted dry mass obtained using ethanol (Me= 0.6467, SD=0.01133) significantly differs from the average value of extracted dry mass obtained using methanol as the extraction solvent (Me= 0.7421, SD=0.0084) with an average difference R=-0.0954800 grams, Sig.=0.000, at a significance level of p = 0.01 in the maceration extraction method (Table 7, Table 8, Figure 3).

Table 7. Descriptive indicators of extracted dry mass of extract after 6 hours of maceration extraction method.

E	N	Me	SD	SE	95% Confidence Interval for Mean		Min.	Max.
					LB	UB		
Water	5	0.9526	0.0136	0.0060	0.9356	0.9695	0.9382	0.9702
EtOH	5	0.6467	0.0113	0.0050	0.6326	0.6607	0.6311	0.6621
MeOH	5	0.7421	0.0084	0.0037	0.7316	0.7526	0.7317	0.7539
Total	15	0.7804	0.1326	0.0342	0.7070	0.8539	0.6311	0.9702
ANOVA			**F(2,14)= 952.649,**		**Sig.=0.000**			

Legend: E - Extraction, Me - Mean, SD - Standard Deviation, SE - Standard Error, 95% CI - 95% Confidence Interval for Mean, Min - Minimum, Max - Maximum

Table 8. Results of subsequent comparison of the average dry mass of extract using Tukey's HSD test for real differences in maceration extraction method after 6 hours.

(I) S	(J) S	MD (I-J)	SE	Sig.	99% Confidence Interval	
					LB	UB
1 Water	2 EtOH	0.3059*	0.0071	0.000	0.2803	0.3314
	3 MeOH	0.2104*	0.0071	0.000	0.1848	0.2360
2 EtOH	1 Water	-0.3059*	0.0071	0.000	-0.3314	-0.2803
	3 MeOH	-0.0954*	0.0071	0.000	-0.1210	-0.0698
3 MeOH	1 Water	-0.2104*	0.0071	0.000	-0.2360	-0.1848
	2 EtOH	0.0954*	0.0071	0.000	0.0698	0.1210

***. The mean difference is significant at the 0.01 level.**

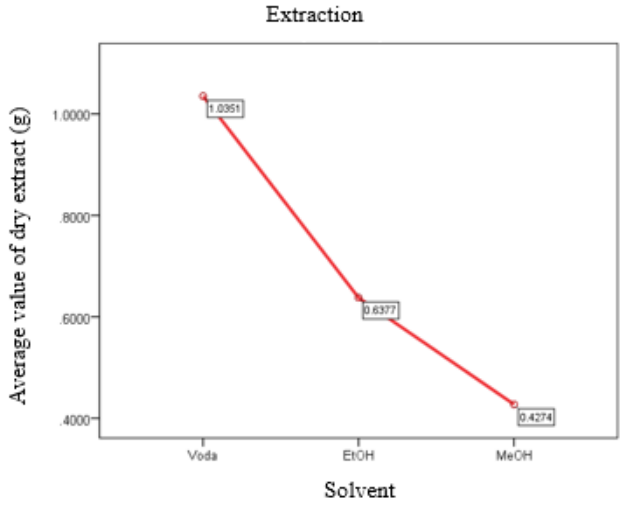

Fig. 3. Average values of extracted dry mass in relation to the type of extraction solvent used after 6 hours.

Subsequent comparison within group g4 - maceration extraction after 8 hours using Tukey's HSD test for real differences showed that the average dry mass value of Milk thistle extract extracted after 8 hours of soaking in water (Me= 0.5103, SD=0.0182) significantly differs from the average dry mass value extracted by ethanol (Me= 0.397000, SD=0.0327) with an average difference, R=0.1133, Sig.= 0.000, at a significance level of p = 0.01, **but does not significantly differ** from the average dry mass extracted by methanol (Me= 0.5490, SD=0.0145) (Table 9, Table 10, Figure 5). Furthermore, the average dry mass of extract obtained by soaking in ethanol (Me= 0.3970, SD=0.0327) significantly differs from the average dry mass obtained by soaking in methanol (Me= 0.5490, SD=0.0145), with an average difference R= 0.1520, Sig.= 0.000, at a significance level of p = 0.01 (Table 9, Table 10, Figure 4).

Table 9. Descriptive indicators of extracted dry mass of extract after 8 hours of maceration extraction.

E	N	Me	SD	SE	95% Confidence Interval for Mean		Min.	Max.
					LB	UB		
Water	5	0.5103	0.0182	0.0081	0.4876	0.5330	0.5008	0.5430
EtOH	5	0.3970	0.0327	0.0146	0.3563	0.4376	0.3635	0.4492
MeOH	5	0.5490	0.0145	0.0065	0.5309	0.5671	0.5342	0.5650
Total	15	0.4854	0.0701	0.0181	0.4466	0.5243	0.3635	0.5650
ANOVA					**F(2,14)= 57.850,**		**Sig.=0.000**	

Legend: E - Extraction, Me - Mean, SD - Standard Deviation, SE - Standard Error, 95% CI - 95% Confidence Interval for Mean, Min - Minimum, Max - Maximum

Table 10. Results of subsequent comparison of the average dry mass of extract using Tukey's HSD test for real differences in maceration extraction after 8 hours.

Tukey HSD						
(I) S	(J) S	MD (I-J)	SE	Sig.	99% Confidence Interval	
					LB	UB
Water	EtOH	0.1133*	0.0146	0.000	0.0609	0.1657
	MeOH	-0.0387	0.0146	0.053	-0.0911	0.0137
EtOH	Water	-0.1133*	0.0146	0.000	-0.1657	-0.0609
	MeOH	-0.1520*	0.0146	0.000	-0.2044	-0.0996
MeOH	Water	0.0387	0.0146	0.053	-0.0137	0.0911
	EtOH	0.1520*	0.0146	0.000	0.0996	0.2044
*. The mean difference is significant at the 0.01 level.						

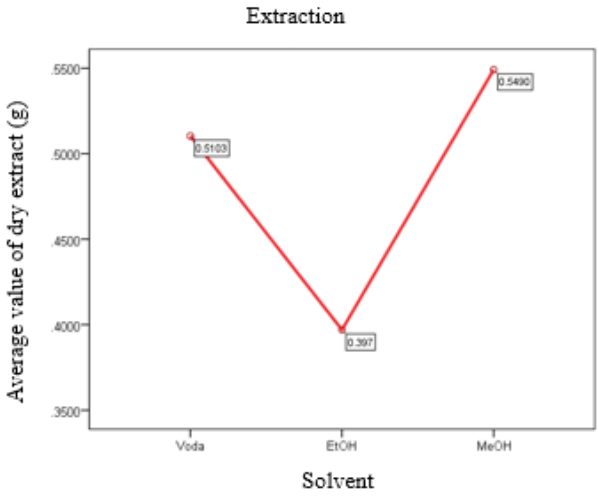

Fig. 4. The average values of extracted dry mass through maceration extraction relative to the type of extraction solvent used after 8 hours.

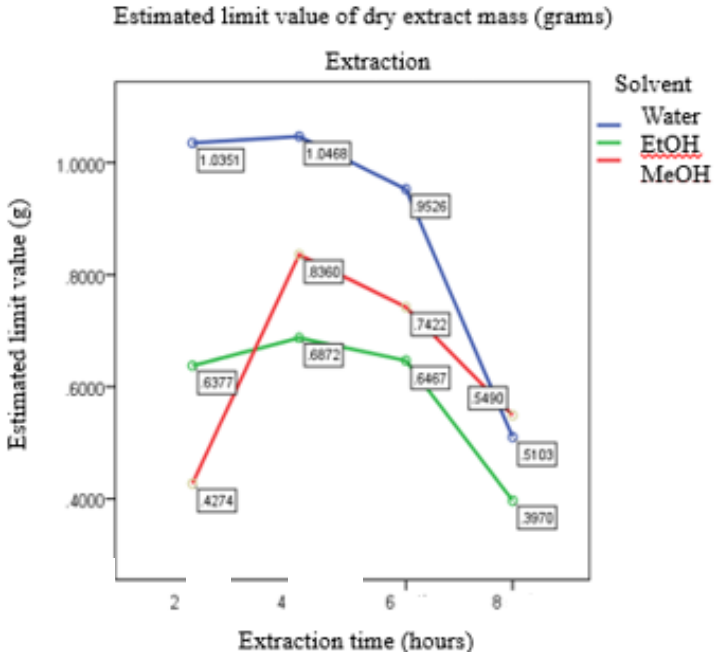

Figure 5. Average values of extracted dry mass of extract according to the type of solvent in relation to the extraction time.

Table 11. Results of applying ANOVA in assessing the influence of extraction time on the average extracted dry mass of Milk thistle in relation to the type of solvent used in the maceration extraction method.

Extraction Time	Solvent Type		
	G1: (Water)	G2:(EtOH)	G3: (MeOH)
2 hours			
4 hours	F(3,19)= 2383.36	F(3,19)= 279.94	F(3,19)= 2181.61
6 hours	Sig.=0.000	Sig.=0.000	Sig.=0.000

Table 12. Average values of extracted dry mass of homogeneous subsets grouped by subsequent comparison using Tukey's HSD test for real differences, isolated when the extraction was conducted using the maceration method and water and ethanol were used as solvents, at a significance level of p=0.01.

ET	N	Water			ET	Ethanol (EtOH)		
		Subset for alpha = 0.01				Subset for alpha = 0.01		
		1	2	1		1	2	3
8	5	**0.5103**			8	0.3970		
6	5		**0.9526**		2		0.6376	
2	5			**1.0351**	6		0.6467	0.6467
4	5			**1.0468**	4			0.6872
S		1.000	1.000	0.412	S	1.000	0.849	0.011

Legend: ET-Extraction Time, S = Sig.,

Table 13. Average values of extracted dry mass of homogeneous subsets grouped by subsequent comparison using Tukey's HSD test for real differences, isolated when the extraction was conducted using the maceration method and methanol was used as the solvent, at a significance level of p=0.01.

Extraction Time hours	Methanol (MeOH)			
	Subset for alpha = 0.01			
	1	2	3	4
2 hours	**0.4274**			4
8 hours		0.5490		
6 hours			0.7421	
4 hours				0.8360
Sig.	**1.000**	1.000	1.000	1.000

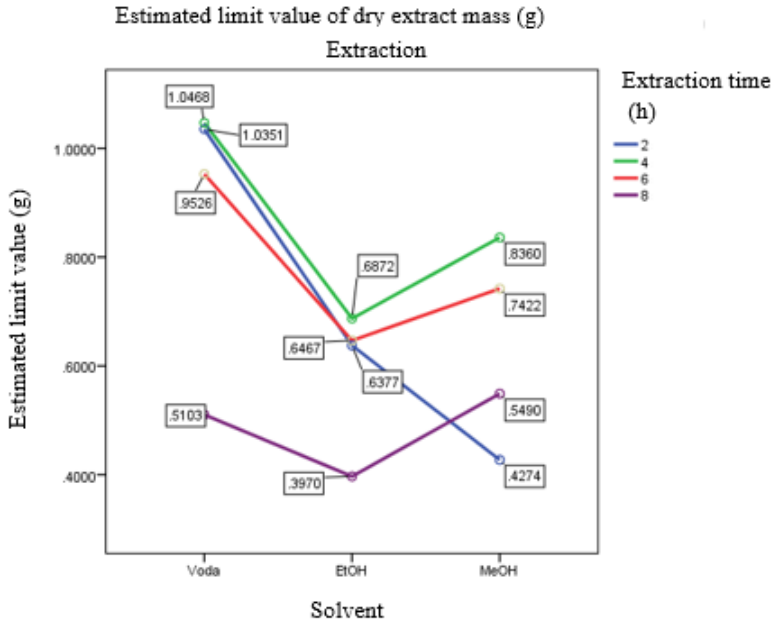

Fig. 6. Average values of extracted dry mass of Milk thistle extract according to extraction time in relation to the extraction solvent.

Table 14. Results of subsequent comparison of the average dry mass of extractusing Tukey's HSD test for real differences in maceration extraction according to the type of solvent and exposure time.

S	(I) ET h	(J) ET h	MD (I-J)	SE	Sig.	99% Confidence Interval	
						LB	UB
Water	2	**4**	**-0.0117**	**0.0073**	**0.412**	-0.0387	0.0153
		6	0.0825*	0.0073	0.000	0.0554	0.1095
		8	0.5247*	0.0073	0.000	0.4977	0.5517
	4	**2**	**0.0117**	**0.0073**	**0.412**	-0.0153	0.0387
		6	0.0942*	0.0073	.000	0.0671	0.1212
		8	0.5364*	0.0073	0.000	0.5094	0.5634
	6	2	-0.0825*	0.0073	0.000	-0.1095	-0.0554
		4	-0.0942*	0.0073	0.000	-0.1212	-0.0671
		8	0.4422*	0.0073	0.000	0.4152	0.4692
	8	2	-0.5247*	0.0073	0.000	-0.5517	-0.4977
		4	-0.5364*	0.0073	0.000	-0.5634	-0.5094
		6	-0.4422*	0.0073	0.000	-0.4692	-0.4152
EtOH	2	4	-0.0495*	0.0111	0.002	-0.0904	-0.0086
		6	**-0.0090**	**0.0111**	**0.849**	-0.0499	0.0318
		8	0.2406*	0.0111	0.000	0.1997	0.2815
	4	2	0.0495*	0.0111	0.002	0.0086	0.0904
		6	**0.0405**	**0.0111**	**0.011**	-0.0004	0.0814
		8	0.2902*	0.0111	0.000	0.2492	0.3311
	6	**2**	**0.0090**	**0.0111**	**0.849**	-0.0318	0.0499
		4	-0.040	0.0111	0.011	-0.0814	0.0004
		8	0.2497*	0.0111	0.000	0.2087	0.2906
	8	2	-0.2406*	0.0111	0.000	-0.2815	-0.1997
		4	-0.2902*	0.0111	0.000	-0.3311	-0.2492
		6	-0.2497*	0.0111	0.000	-0.2906	-0.2087
MeOH	2	4	-0.4086*	0.0055	0.000	-0.4291	-0.3880
		6	-0.3147*	0.0055	0.000	-0.3353	-0.2942
		8	-0.1216*	0.0055	0.000	-0.1421	-0.1011
	4	2	0.4086*	0.0055	0.000	.03880	0.4291
		6	0.0938*	0.0055	0.000	0.0732	0.1143
		8	0.2869*	0.0055	0.000	0.2664	0.3074
	6	2	0.3147*	0.0055	0.000	0.2942	0.3353
		4	-0.0938*	0.0055	0.000	-0.1143	-0.0732
		8	0.1931*	0.0055	0.000	0.1726	0.2136
	8	2	0.1216*	0.0055	0.000	0.1011	0.1421
		4	-0.2869*	0.0055	0.000	-0.3074	-0.2664
		6	-0.1931*	0.0055	0.000	-0.2136	-0.1726

Legend: S = solvent, ET = Extraction Time, h = hours,

CONCLUSION

Based on the conducted comparisons, we conclude that the highest average mass of dry extract extracted by **maceration** was achieved by **soaking in water**, with an extracted mass in five repetitions after **4 hours** averaging Me=1.0468 **g**, followed by extraction after 2 hours with an average extracted mass of **Me=1.0351 g**, and extraction after 6 hours with an average extracted mass of Me=0.9526 g (Table 1, Figure 5).
When extraction was performed after 8 hours, the average mass of dry extract extracted in five repetitions with **methanol** as the solvent, Me=0.5490, does not significantly differ from the average mass of dry extract extracted by soaking in **water**, Me=0.5103 (Table 1, Figure 5). In maceration extraction performed after 2 hours, the average mass of dry extract of milk thistle seeds (*Silybum marianum*) extracted using ethanol, Me=0.6377, was higher than that extracted by methanol, Me=0.4274 g, while the lowest yield by maceration occurred using methanol for extraction after 4, 6, and 8 hours, with average yield values ranging from Me=0.6872 g to Me=0.3970 g (Table 1, Figure 5).

Subsequent comparison using Tukey's HSD test revealed that the average values of dry extract mass extracted in five repetitions using the following solvents:

- *water* significantly differ from each other and can be grouped into three homogeneous groups. One group consists of the extracted mass Me=0.5103 g after 8 hours, the second group consists of the average mass of Me=0.9526 g extracted after 6 hours, and the third group consists of the average values of Me=1.0351 grams extracted after 2 hours and Me=1.0468 grams extracted after 4 hours, where the highest average yield was achieved, at the level of statistical significance p=0.01 (Table 12, Table 14, Figure 6).
- *ethanol* significantly differ from each other and can be grouped into three homogeneous groups. One group consists of the average mass of dry extract extracted after 8 hours: Me=0.3970 g, the second group consists of the extracted dry extract masses of milk thistle after 2 and 6 hours: Me=0.6376 and Me=0.6467 g, and the third group consists of the average values of dry extract mass extracted after 6 hours: Me=0.6467 g and 4 hours: Me=0.6872 g, where the highest average value for ethanol was obtained, at the significance level p=0.001 (Table 12, Table 14, Figure 6).
- *methanol* significantly differ from each other and can be grouped into four homogeneous groups. Each group consists of the average mass of dry extract of milk thistle extracted in the following order: after 2 hours Me=0.4274, after 8 hours: Me=0.5490 g, after 6 hours: Me=0.7421, and after 4 hours, Me=0.8360 g where the highest yield was achieved (Table 13, Table 14, Figure 6).

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