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CONTENT


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EFFECT OF EXTRACTION TECHNIQUE ON THE CONTENT OF BIOACTIVE COMPONENTS AND ANTIOXIDANT ACTIVITY OF AQUEOUS EXTRACTS OF FRESH AND DRIED NETTLE (*URTICA DIOICA* L.)

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:
The samples of stinging nettle were collected during June in the Tuzla region. Aqueous extracts were prepared from fresh and dried leaves in order to determinate and compare content of bioactive components and antioxidant potential. Conventional soxhlet, ultrasound assisted extraction and traditional maceration extraction were used as extraction methods. Quantitative determination of phenols and flavonoids was carried out using spectrophotometric methods. Antioxidant activity of nettle aqueous extracts was determined using ferric reducing antioxidant power and DPPH free radical scavenging activity. Extracts obtained by Soxhlet extraction showed the highest total phenolic and flavonoid content and expected the highest antioxidant capacity, while extracts obtained by maceration gave the lowest results.

KEYWORDS: stinging nettle extract; bioactive components; extraction; antioxidant

INTRODUCTION

Stinging nettle (*Urtica dioica* L.) is a widespread perennial herbaceous plant. Urtica dioica and the closely related Urtica urens, as the most prominent members of the genus Urtica, are native to Europe, Africa, Asia and North America [1]. Eastern Germany, the former USSR, Bulgaria, the former Yugoslavia, Hungry and Albania are known as primary producers of stinging nettles [2]. Nettle, as one of the most undervalued of economic plants, has a long history of use as food, dye, cosmetic, and drug in folk veterinary and human medicine. It is widely recognized and used as an edible and highly nutritious vegetable, from young leaves that are added to soups or salads to dried leaves for winter use [3-5]. Beside its usage in human nutrition, nettle has been used all over the world for centuries in traditional medicine. This plant was used for the treatment of arthritis, anemia, rheumatism of the joints and muscles, hypertension, gastrointestinal diseasas, eczema and used as diuretics, astrigents, cleansing tonic, component of antidiabetic teas and blood purifier [6-10]. Studies showed the presence of different class of compounds in nettle including polyphenols, flavonoids, phenolic acids, alkaloids, carotenoids, choline, histamine, macro and microelements [7, 11-14]. Plants produce many chemical compounds, as a result of various biochemical pathways, that are biologically active. These components are found throughout the plant world and they can be shown to have an effect on human health. The presence of flavonoids and phenolic acids, as two major classes of phenolic compounds, enables plants to act as reducing agents, hydrogen donators and signlet oxygen quenchers. When plant posses high amounts of these compounds it has potent antioxidant activity leading to various defensive and disease fighting properties [15]. Lutein, β-carotene and their isomers were found to be the major carotenoids during all leaf maturity levels [5]. Nettle is also good source of vitamins C, K, and B groups vitamins and minerals, such as calcium, iron, magnesium, phoshorus, pottasium and sodium [2, 4]. Due to its content of bioactive components nettle has been subject of study for antioxidant, anti ulcer and anaglesic properties, which were confirmed to be present [16].

The aim of this study is to determine and compare a content of biological components and antioxidant power of aqueous extracts derived from fresh and dried leaves of stinging nettle. There are available other studies that focus on aqueous extracts of Urtica dioica, from investigating biological compounds and antioxidant capacity to antihyperglycemic activity, diuretic and hypotensive effects [17-19].

It is important to mention that all these studies were done in different parts of the world, on different types of nettle extract and various experimental methods were applied on them. Factors such as type of extraction method, type of compounds and solvents, extraction efficiencies, the geographical and ecological status, the climate, seasonal and experimental conditions and other, all play a role and may explain the diversity of results in different studies [1]. This can be especially noticed in significant differences of the results in studies that showcase antimicrobial activity of nettle. Some studies revealed that nettle extracts had inhibitory effects on various Gram positive and Gram negative bacteria, while some noted that ethanol, methanol and organic solvent extracts were antimicrobial inactive [16, 20-24].

EXPERIMENTAL

All chemicals used were of analytical grade and were used as received without any further purification. Chemicals were purchased from Merck (Darmstadt, Germany) and Sigma Chemical Co. (St. Louis, Missouri, USA).

SAMPLING AND PREPARATION OF MATERIALS FOR ANALYSIS

A nettle sample was harvested in the Tuzla area in June. The sample was cleaned and the leaves separated from the rest of the plant. The fresh leaves was immediately subjected to extraction. Part of the fresh leaves was dried for seven days at room temperature, in a dark and dry place. Fresh and dried nettle samples were separately ground in an electric mill before the extraction process.

PREPARATION OF EXTRACTS

Three methods were used for extraction: Soxhlet extraction, ultrasonic extraction and maceration. In all three cases, 10 grams of chopped fresh or dried nettle were weighed and transferred to a flat-bottomed balloon, or paper tube (in the case of Soxhlet extraction), and poured with 150 mL of methanol. Ultrasonic extraction was performed in an Elmasonic S ultrasonic bath, without heating. Maceration was performed at room temperature with stirring at 300 rpm with Tehtnica Vibromix 40. After four hours of extraction, the extracts were filtered through filter paper. The obtained filtrates were stored in a dark and cool place before analyzing.

DETERMINATION OF TOTAL PHENOLIC CONTENT (TPC)

Total phenolic were determined through the Follin-Ciocalteu test following the protocol [25] with some modifications. 200 μL of extracts was mixed with 2.54 mL of 10% Folin-Ciocalteu reagent. After 5 min 420 μL of 10% sodium carbonate was added. After incubation for 60 minutes at room temperature, the absorbance was measured at 765 nm, along with the prepared blank. Gallic acid was used as standard for the calibration curve ($y= 0{,}0042x + 0{,}0076$, $R^2 = 0{,}9998$). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrammes per 100 grams of nettle.

DETERMINATION OF TOTAL FLAVONOID CONTENTS (TFC)

Total flavonoid content in the extracts was measured by the previously described method [26], with some modification. 1mL of extract solution were mixed with 0.3 mL of 5% sodium nitrate. 0.3 mL of 10% aluminium chloride was added after 5 minutes. After 6 minutes incubation at room temperature, 1 mL of 1 M sodium hydroxide was added to the reaction mixture. Immediately the final volume was make up to 10 mL with distilled water. Absorbance of sample was determined against the blank at 510 nm using a spectrophotometer. The results were derived from the calibration curve ($y = 3{,}024x – 0{,}0034$; $R^2 = 0{,}9984$) of quercetin and expressed in quercetin equivalents (QE) per 100 grams of sample (mg/100 g) .

FERRIC-REDUCING ANTIOXIDANT POWER (FRAP) ASSAY

The reducing power of the extracts, that reflected their antioxidant activity, was determined according to the protocol [27]. 3 mL of prepared FRAP reagent was mixed with 100 μL of diluted extracts. Absorbance at 593 nm was recorded after a 30 min incubation at 37˚C. The FRAP value was calculated from the calibration curve of iron(II) sulfate heptahydrate ($y = 0{,}001x + 0{,}0698$; $R^2 = 0{,}9997$) and expressed in mol per gram of nettle sample.

DPPH RADICAL SCAVENGING ACTIVITY

2,2-diphenyl-1-picryl-hydrazyl (DPPH) method was performed according to earlier described method [28]. A series of solutions was made in tubes by adding different volumes of extract supplemented with up to 2 mL of methanol. 0.5 mL of 0.5 mM DPPH solution were added and the samples were left to incubate for 30 minutes in a darkened room at a room temperature. The absorbance was measured at 517 nm along with a methanol as blank sample. 0.5 mL of 0.5 mM DPPH dilution, diluted with 4 mL of methanol, was used as a control sample. The radical scavenging effect (%) or percent inhibition of DPPH radical was calculated according to the equation:

$$AA\% = [(Ac - As) / Ac] \times 100$$

where As is the absorbance of the solution containing the sample at 517 nm and Ac is the absorbance of the DPPH solution. The results are expressed as the IC_{50} value (mg/mL).

DETERMINATION OF BIOELEMENT CONTENT IN PLANT MATERIAL AND EXTRACTS

3 grams of dried nettle leaves material were topped with aqua regia and incubated for 16 hours. After 16 hours, the mixture was heated to reflux for 2 hours. The content of bioelements in such prepared parent sample was determined using ICP OPTIMA 2100 DV (Perkin Elmer) spectrometer. Aqueous extracts of plant material were directly analysed, withour prior preparation.

RESULTS AND DISCUSSION

Table 1 shows the results of the content of total phenolic and flavonoids (designated by U) in the aqueous extracts of fresh and dried nettle, obtained by three extracting techniques: conventional soxhlet, ultrasound assisted extraction and traditional maceration extraction (designated by numbers 1, 2 and 3). The notation (f) indicates the fresh sample, and (d) marks the dried sample. The results showed that extracts obtained by Soxhlet extraction have the hightest total phenolic and flavonoid content per gram of plant sample, for both – fresh and dry samples, while extracts obtained by maceration have the lowest content, leaving extracts derived from ultrasonic extraction in the middle of this rank. The Soxhlet extraction, as conventional technique, has been widely used for the extraction of bioactive compounds from various plant materials [29]. Within conventional methods applied for the Extraction of Bioactive Compounds from Lebanese Urtica dioica, it was found that soxhlet method had the highest extraction yield and the maceration method had the lowest one [30]. The higher total phenolic and total flavonoids content in the extracts obtained by Soxhlet extraction, compared to those obtained by maceration and ultrasonic extraction, is probably due to the solvent circulation flow until the complete depletion of plant material [31].

It is important to note that aqueous extracts of dried nettle samples showed higher values of total phenols and flavonoids compared to extracts obtained from fresh samples. This may be related to the fragility of fresh samples, which tend to deteriorate faster than dry samples [32]. Air drying at ambient temperature was used in many studies of nettle bioactive compounds for samples preparation [33-37] since does not force dried plant materials using high temperature; hence, heat-labile compounds are preserved [38].

Table 1. Content of total phenolic and flavonoids in nettle extracts

Extract	TPC [mgGAE/1g]	TFC [mgQE/1g]
U-1(f)	14,34	$9{,}52 \times 10^{-3}$
U-2(f)	11,67	$6{,}28 \times 10^{-3}$
U-3(f)	2,84	-
U-1(d)	63,59	$25{,}77 \times 10^{-3}$
U-2(d)	26,61	$14{,}98 \times 10^{-3}$
U-3(d)	12,23	$4{,}28 \times 10^{-3}$

Total phenolic and flavonoid contents of aqueous extract of stinging nettle have been studied earlier. The total phenolic content obtained through study published in 2014. were at 322.941±11.811 mgGAE/g extract value, while total flavonoids were 133.916±12.006 mg Catechin/g extract [15]. Reason why results are different from presented above is probably due to different method of extracting. Aim of this study was to compare results of three different extraction methods and of two types of samples, extracts of fresh and shade dried and to ease the process of choosing extraction technique.

ANTIOXIDANT ACTIVITY

Table 2. Results of antioxidant capacity and reduction ability of extracts obtained by DPPH and FRAP method

Extract	DPPH IC_{50} [mg/mL]	FRAP value [µmol/g]
U-1(f)	0,744	12171
U-2(f)	0,918	11257
U-3(f)	12,67	277,37
U-1(d)	0,122	61856
U-2(d)	0,145	46344
U-3(d)	0,292	9598,5

Antioxidant activity of aqueous extracts derived from nettle was determined using FRAP and DPPH methods, and results of antioxidant capacity are shown in Table 2. Instead of costly and inefficient separation of each phenolic antioxidantan, total antioxidant power of a complex sample is often more meaningful to evaluate the health benefits, because of the cooperative action of antioxidants [39].

Extracts that were obtained by maceration had the lowest antioxidant capacity, while extracts obtained with Soxhlet extraction had the highest antioxidant capacity. The above data correspond to the content of total phenolics which are responsible for the antioxidant capacity of the samples. A significant correlation between the content of phenolic compounds in plants and the antioxidant capacity has been observed in many studies [40-44].

Once more, it was confirmed that extracts of dried nettle showed higher antioxidant capacity. To compare, Vitamin C with IC_{50} 0,03 mg/ml has much higher antioxidant capacity than obtained extracts by the present study. The nearest value to IC_{50} of Vitamin C is the value of DPPH noted in aqueous extract from shade dried nettle obtained by Soxhlet extraction. The antioxidant power of nettle extracts in present study can also be related to their phenolic content. This can be explained by the larger contact area between the dry sample and the extraction solvent. Due to the smaller and more homogeneous particle size of dried samples, more reducing compounds could be detected by extraction of dried leaves, than in the case of fresh plant material [33].

In earlier mentioned study [15], the IC_{50} of DPPH radical was 1.2 mg/mL. Even though values of total phenolics and flavonoids were higher, the above mentioned IC_{50} value is higher of every value in Table 2 of present study, except of DDPH result for extraction of fresh nettle obtained from maceration.

BIOELEMENTS CONTENT

Table 3 show the results of the content of bioelements in the extracts and in the parent sample which is listed in the table as nettle leaf. According to American Herbal Pharmocopoeia [45], the leaves of *Urtica dioica* are rich in mineral such as calcium, iron, magnesium, phosphorus, potassium and sodium, which is also confirmed by the results of analyzed elements in present study.

Table 3. Content of bioelements in extracts derived from netttle material and in parent sample (mg/kg)

Element	U-1(f)	U-2(f)	U-3(f)	U-1(d)	U-2(d)	U-3(d)	Nettle leaf
Fe	0,69	0,84	1,065	0,24	1,68	2,055	113,96
Cu	1,215	0,33	0,285	0,405	1,125	2,37	2,70
Co	0,045	0,045	0,06	0,06	0,06	0,06	0,10
Ca	3160,5	5037	4440	17115	12148,5	9009	17966,6
Na	144,6	91,2	125,4	283,05	114,45	174,7	381,00

In this study, the content of bioelements in extracts obtained from dry samples of nettle, in comparison with raw samples, generally had values closer to those in the parent sample. Figures 1 and 2 graphically show the extraction efficiency of bioelements from fresh and dried nettle leaves.

Among the three applied methods of extraction from dry nettle samples, soxhlet gave extracts with the contents of macroelements Ca and Na that are closest to those in the parenting sample. In other study [46], a higher content of macroelements Ca and Na was extracted from dried nettle leaves using maceration, than by Soxhlet and ultrasound-assisted techniques. Still, all three extraction methods in present study showed a significantly higher calcium content compared to sodium, in both fresh and dried samples, which is consistent with other reports of nettle, where calcium dominates compared to other macrominerals [3, 36, 47-48]. In addition to the highest content of macroelement calcium, the highest content among the analyzed microelements of the parent sample of nettle leaves had iron. In the comparison research of nutritional properties of Stinging nettle (Urtica dioica) powder with wheat and barley powders, calcium and iron levels in nettle powder were much higher than those from wheat and barley powders [49].

However, with regard to the extraction of Fe and Cu microelements in present study, maceration was more efficient than other applied methods. The results of the presence of cobalt in this study, both in the parent sample and in all obtained extracts from fresh and dried nettle leaves, showed the presence of cobalt in a lower content compared to other analyzed heavy metals, ie iron and copper. The order of Fe, Cu and Co content in nettle samples of this study is consistent with that in the study of concentrations of heavy metals in 54 samples of individuals and mixtures of medicinal herbal plants [50] where the order of decreasing mean total concentrations for these metals was Fe > Cu > Co, and cobalt was the least detected element.

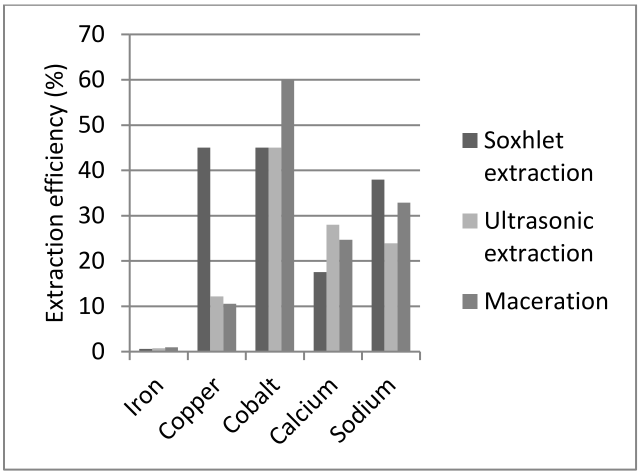

Figure 1. Efficiency of bioelements extraction from fresh netttle leaves by different extraction techniques

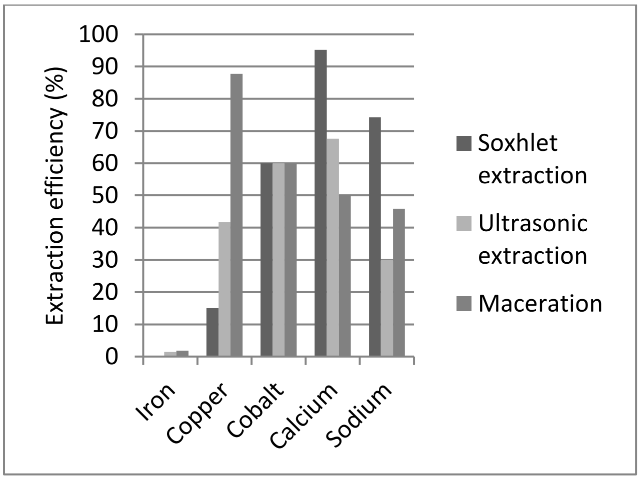

Figure 2. Efficiency of bioelements extraction from dried netttle leaves by different extraction techniques

CONCLUSION

Nettle leaf extracts showed different contents of bioactive compounds and bioelements, depending on the type of plant sample subjected to extraction, i.e. fresh or dry, as well as the extraction technique applied. In presented study, dry nettle leaf samples had yielded extracts with higher content of both bioactive compounds and bioelements, compared to fresh samples, regardless of the extraction method that has been used. Extracts obtained by Soxhlet extraction showed the highest total content of phenols and flavonoids, and accordingly the highest antioxidant capacity, while extracts obtained by maceration gave the poorest results. Conventional extraction techniques generaly have yielded extracts with a higher content of bioelements, where the Soxhlet method was more efficient in extracting macroelements Ca and Na from plant material, while maceration yielded extracts with a higher content of Fe and Cu microelements. The results of this research may contribute to the selection of the appropriate method of nettle samples preparation for extraction, as well as the appropriate extraction method, depending on the target bioactive compounds or bioelements in the plant material.

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PHYSICOCHEMICAL CHARACTERISTICS OF PRODUCTS BASED ON HONEY AND HAWTHORN (*CRATAEGUS SPP.*)

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:
Medicinal plants have become an increasing subject of interest worldwide due to the large amount of biologically active substances that have potential beneficial health properties. One of the more interesting popular medicinal plants is hawthorn (*Crataegus spp.*), a deciduous branched shrub that is increasingly used for health purposes. Various parts of this plant, including berries, flowers and leaves, are rich in nutrients and beneficial bioactive compounds that are effective in the treatment of numerous diseases. Honey is a natural sweetener produced by bees from plant secretions. Known for its nutritional and medicinal values, it gives strength to the body, which is why it is indispensable in the human diet. The combination of these two ingredients represents a significant source of vitamins and minerals in daily use, but also for use in various pharmaceutical and medical purpose. Taking into account all of the above, the aim of the work is to test samples of the mixture of honey and hawthorn in different proportions and determine the physical and chemical characteristics: pH value, electrical conductivity, refractive index, viscosity, water activity, HMF, DPPH. Based on the analysis, appropriate conclusions will be drawn and more information will be obtained about their quality and possible use as a food supplement. On the basis of the conducted analyses, it can be concluded that the parameter values are within the permitted limits defined by the Rulebook (Official Gazette Bosnia and Herzegovina No. 37/09). The analysis of the mentioned parameters showed that the chemical composition of the sample plays a major role in the value of the measurement results, and that Sample III has the best antioxidant properties.

KEYWORDS: physicochemical characteristics; honey; hawthorn (*Crataegus spp.);* mixture; food suplement

INTRODUCTION

Hawthorn (*Crataegus spp.*) is a shrub or tree with ramified branches. It belongs to the family Rosaceae, subfamily Maloideae, genus Crataegus.[1] It is widely distributed in Asia, Africa, North America and Northern Europe, and there are 165-200 species in the world. [2] The chemical composition of hawthorn depends on the part of the plant, so we distinguish the chemical composition of the flower, leaf or fruit. The ripe fruit has the highest concentrations of glucose and fructose, while the hawthorn leaf and flower contain sugars and sugar alcohols, organic acids, terpenes, plant essential oils, phenylpropanoids, hydroxycinnamic acids, lignans and flavonoid oils.[3] Various parts of this plant, especially the berries, flowers and leaves are rich in nutrients and traditionally associated with many health, medicinal or pharmaceutical positive effects on health, e.g. antimicrobial, anti-inflammatory, antioxidant, anticancer and anticoagulant action, which is why this plant has been used as a traditional medicine, herbal remedy and food supplement for a long time. For example Hawthorn fruit has long been used as a functional food in China due to its effects of increasing appetite, promoting digestion and protecting the gastrointestinal tract. [4] Unlike other common fruits, hawthorn has more dietary fiber, pectin, ascorbic acid, minerals and antioxidant capacity, which is why it has been approved as a medicinal fruit by the Chinese National Health Commission.[5],[6] Studies have confirmed that hawthorn is rich in amino acids (8 essential amino acids and 3–8 times more amino acids than fruit), protein (17 times more protein than apple fruit), sugars, minerals (1st in calcium content among fruits), vitamins (vitamins A, C, B1, B2, about 10 times more vitamins), and has a high nutritional value. [7]

Honey is a naturally sweet product produced by honey bees (Apis mellifera) from nectar of honeydew plants, secretions of living parts of plants or secretions of insects that suck living parts of plants, which bees collect, add their specific substances, store, excrete water and deposit in the cells of the comb until maturation. [8] Chemically, honey is a very complex mixture of more than 70 different ingredients. [9] Different types of honey, as well as honey within one type, differ in their composition depending on the sort of plant and geographical origin, climatic conditions, type of bees and the ability of the beekeeper himself. The most common ingredients are carbohydrates, mostly fructose and glucose, and water, which together make up more than 99% of honey. [10] The rest consists of proteins (including enzymes), mineral substances, vitamins, organic acids, phenolic compounds, aroma substances (volatile compounds) and various chlorophyll derivatives, which is why it gives strength, gives strength to the body and ensures physical endurance and psychological stability.

Because of all the above, the combination of these two ingredients represents a significant source of vitamins and minerals in daily use, but also for use in various pharmaceutical and medical purposes. In order to obtain specific data of products based on honey and hawthorn, the purpose of the work is the analysis of physical and chemical parameters such as: pH value, electrical conductivity, viscosity, refractive index, water activity, HMF, DPPH. As the literary data is scarce when it comes to the combination of these two natural ingredients, the aim of the analysis is to determine the physical and chemical characteristics of products based on honey and hawthorn in different proportions, in order to obtain more information about their quality and possible use as a food supplement.

MATERIAL AND METHODS

MATERIAL

For the research, three samples of honey and hawthorn (*Crataegi folium cum flore*) of different proportions with a total weight of 50 g were prepared. Meadow honey bought in a supermarket was used. Hawthorn was ground into powder and mixed with honey in the following proportions:

Table 1. Content of used sample

Name of sample	Content
Sample I	5g Hawthorns + 45 g honey
Sample II	10 g Hawthorns+40 g honey
Sample III	20 g Hawthorns + 30 g honey

The prepared samples were left to stand for 10 days after which they were used for analysis.

METHODS

Determination of the pH value was performed using a Mettler Toledo 220 pH meter. The measurement is based on immersing the electrode of the pH meter in the sample, with prior calibration of the instrument with exactly known standard solutions. Electrical conductivity was determined with the help of a conductometer, also with previous calibration of the instrument with accurately prepared standard solutions. The refractive index for the mentioned samples was determined with the help of an Abbe refractometer, while the water activity was determined with the help of determination water activity device.

Viscosity was determined using an Ostwald viscometer at temperatures of 25°C (room temperature) and 40°C. From each sample, a mixture was prepared in such a way that 5 g of the sample was weighed and dissolved in 95 ml of water, and then measurements were made at the given temperatures. The density was determined using a pycnometer, and the viscosity value was calculated according to the following relation:

$$\eta_1 = \eta_2 \frac{\rho_1 \cdot t_1}{\rho_2 \cdot t_2} \quad \text{...} \quad (1)$$

where is:
η_1-viscosity of sample;
η_2-viscosity of destilled water;
ρ_1-density of sample;
ρ_2-density of destilled water;
t_1- time of sample;
t_2-time of destilled water.

The hydroxymethylfurfural content was determined by the White method at two wavelengths, 284 and 336 nm. The measurement for the samples and the standard was performed at 284 and 336 nm, after which the *HMF* value was calculated according to the following relation:

HMF (mg/kg)= (A_{284} – A_{336}) x 149 x 5 x D/W....... (2)

$$\left(149{,}7 = \frac{126 \cdot 1000 \cdot 1000}{16830 \cdot 10 \cdot 5}\right)$$

where is:
126 - is the molecular weight of HMF;
16,830 molar absorbance at 284 nm;
1000 is the conversion of grams to kg;
0.5 is the theoretical weight of the sample;
D is the dilution factor if dilution is necessary:
W is the weight of the honey sample in g.

The determination of DPPH was performed in ethanol solution because it facilitated the extraction of antioxidant compounds from the sample. 1g was taken from each sample and ethanol, acetate buffer (pH 5.5) and DPPH reagent were added, after which the measurement was performed on a spectrophotometer at a wavelength of 517 nm. Along with the samples, a blank test measurement was also performed.

RESULTS AND DISCUSSION

The physical properties of honey and hawthorn are closely related to the chemical composition of honey. Due to differences in the composition of honey, the values of these parameters can be specific and different.[11] It has been proven that viscosity, refractive index and specific mass depend on the proportion of water. Optical activity is related to the composition and content of certain carbohydrates, while electrical conductivity primarily depends on the content of mineral substances.[12]

Table 2. Values of physicochemical caracteristic products of the analyzed samples

Name of sample	pH	κ, μS/cm	Index of refraction	Water activity	Viscosity N·s/m²		HMF mg/kg
					25°C	40°C	
Sample I	3.88	4.80	1.4978	0.557	933.440	761.622	2.6
Sample II	4.03	3.53	1.5284	0.545	970.583	780.88	6.4
Sample III	4.10	2.16	1.6325	0.508	980.281	814.70	7.1

The analysis of the samples showed that the highest measured pH value was sample III (4.10), while the lowest pH value was sample I (3.88), which shows that the chemical composition plays a big role. The pH value of hawthorn is acidic, which has been confirmed by other authors.[13] Generally, the pH value of honey ranges from 3.2 to 4.5 [14] and is not directly related to free acidity, due to the buffering properties of phosphates, carbonates and other mineral salts, which are naturally present in honey, which further implies that the obtained values of samples with honey content are in accordance with the literature. The lowest value of electrical conductivity was sample III (2.16 μS/cm), while sample I had the highest value (4.80 μS/cm). The electrical conductivity of honey depends on the content of minerals, organic acids, proteins, as well as complex compounds in honey. The electrical conductivity of honey, due to the high concentration of sugar, reduces the mobility of ions, and the electrical conductivity is also lower.[15]

Water activity is a physical property that shows the amount of free water available for the metabolism of the microorganisms present. Many types of bacteria grow at a water activity value (aw) of 0.94 - 0.99, that is, for most yeasts around 0.88, and for mold 0.75 - 0.80. The value of water activity is influenced by factors such as temperature, pH of the environment, content of added salt and others. [16] Sample analysis showed that sample I (one) had the highest water activity value of 0.557 and sample III had the lowest value of 0.508. The average activity of water in honey ranges from 0.56 to 0.62 [17], which shows that the analyzed samples based on honey and hawthorn have values that correspond to the average values of water activity for honey.

By measuring the refractive index, it was determined that sample III had the highest refractive index of 1.6325, while sample I had the lowest refractive index of 1.4978.

Viscosity is the degree of liquidity, i.e. the liquid state, and it particularly affects the processing and storage flow. Viscosity is affected by several factors such as the composition of honey (mainly water content), type of honey, temperature and the number and size of crystals in honey. As the water content increases, the viscosity decreases. As the temperature increases with a constant proportion of water, the viscosity of honey decreases. A higher proportion of di- and trisaccharides contributes to higher viscosity.[18] Along with the water content, temperature has the greatest influence on viscosity.[19] As the temperature increases, the viscosity of honey decreases, and this effect is most pronounced at temperatures below 15 ºC. When determining the viscosity, sample I showed the lowest viscosity, and as the temperature increased, its viscosity further decreased. With the increase in the percentage of honey in the samples, the viscosity also increased, but with the increase in temperature, it decreased in each sample.

Hydroxymethylfurfural (HMF) is a cyclic aldehyde and is naturally present in honey. Its content in fresh honey is very low and amounts to less than 1 mg/kg. The increase in its value is influenced by various parameters such as the use of metal containers for storage, exposure to UV radiation, mineral composition, content of organic acids and moisture, etc. [20] The temperature also has a great influence on the value of HMF, the content of HMF increases rapidly if the temperature of the environment is above 20°C. According to research, the time required for the formation of 30 mg/kg of HMF in honey at a temperature of 30 °C is up to 300 days, and at a temperature of 80ºC it takes less than 2 hours to achieve the same amount of HMF. [21],[22],[23] According to the Ordinance on honey and bee products (Official Gazette Bosnia and Herzegovina No. 37/09), the value of HMF must not exceed 40 mg/kg, which further shows that in the analyzed samples, which are based on a mixture of honey and hawthorn within borders. Notably, the sample with the highest amount of honey had the lowest proportion of HMF.

When determining the antioxidant capacity with the DPPH method, different observations were recorded. With the increase in sample concentration, the ability to capture free radicals also increased. When we compare all three samples, we notice that with the increase in the percentage their antioxidant capacity also increased. Thus, we can say that the third mixture with the highest percentage of hawthorn showed the best ability to trap free radicals. Therefore, we can conclude that the third mixture has the best antioxidant properties.

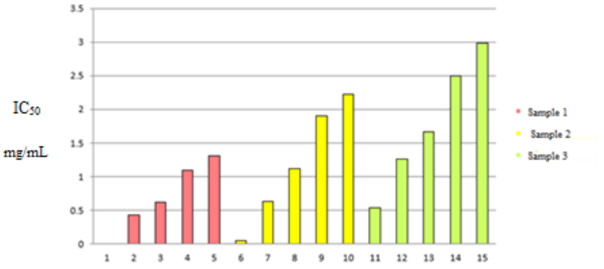

Figure 1. Radical scavenging ability of analyzed samples

CONCLUSION

The analysis showed that the highest pH value was sample III (4.10), the lowest sample I (3.88), which shows that the chemical composition plays a big role. Sample I had the highest electrical conductivity value (4.80 µS/cm), and sample III had the lowest value (2.16 µS/cm). Analysis of the samples showed that sample I had the highest water activity value of 0.557, and sample III had the lowest value of 0.507. The average water activity in honey ranges from 0.56 to 0.62, which shows that the analyzed samples based on honey and hawthorn have values that correspond to the average values of water activity for honey.

By measuring the refractive index, it was determined that sample III had the highest refractive index of 1.6325, while the sample had the lowest refractive index of 1.4978. Sample I showed the lowest viscosity, where as the temperature increased, its viscosity further decreased. With the increase in the percentage of honey in the samples, the viscosity also increased, but with the increase in temperature, it decreased in each sample.

The low value of HMF showed that it is this year's honey, and the obtained values are in accordance with the Ordinance on honey and bee products (Official Gazette of FBiH No. 37/09). The analyzed DPPH value showed that with the increase in sample concentration, the ability to capture free radicals also increased, and that the third mixture with the highest percentage of hawthorn showed the best ability to capture free radicals and the best antioxidant properties.

From the aspect of use and the analyzed physical chemical parameters, sample III showed the best antioxidant properties, but due to its taste consistency, it would be required for use. Sample II also showed good antioxidant properties, easier mixing, and therefore consumption, which would make this sample an excellent choice as a food supplement.

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Influence of starter and sweetener on the syneresis intensity of fermented milk drink

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:
Syneresis represents the appearance of separation of whey on the surface of the product and is considered an important parameter of the quality of fermented dairy products. For the production of the fermented milk drink, kombucha was used as a non-traditional starter. Two sweeteners were used, honey and sucrose. The aim of the work was to examine the influence of starter and sweetener on changes in the subsequent acidity and intensity of syneresis of the samples during storage. The results were compared with a control sample obtained by inoculation with a conventional starter culture. During storage, there was a drop in pH value and an increase in titratable acidity, as a result of the constant metabolic activity of the microflora and the production of lactic acid in the produced samples. Honey, as a sweetener, had an effect on the lower pH value of the fermented milk drink samples. The analysis of variance showed a statistically significant difference in the titratable acidity results and the intensity of syneresis of the samples during storage. Samples that were produced with kombucha as a starter showed better structure stability during storage and better quality. During storage, sample kb4 showed the best structural stability and the lowest intensity of syneresis.

KEYWORDS: stinging nettle extract; bioactive components; extraction; antioxidant

INTRODUCTION

Fermented dairy products are obtained by fermenting milk with lactic acid bacteria, yeasts, but it is also possible to use non-conventional starters such as komucha. In their research, many authors used kombucha for milk fermentation and the production of fermented milk products [1, 2, 3, 4, 5, 6, 7]. *Kombucha* is a symbiotic community of fungi and acetic acid bacteria, which, through metabolic activity on sweetened tea, produces a pleasant, slightly acidic drink that has many beneficial effects on human health. In addition to sucrose, other sweeteners, such as honey, can be used.

Honey has a long history of direct consumption in human nutrition, but it is also often used as a sweetener in many products to improve the aroma and structure of the product. This natural food has long been known for its nutritional and therapeutic aspects. The most important ingredients of honey are carbohydrates in the form of fructose, glucose, disaccharides and oligosaccharides, and the sweet taste of honey is given by components such as maltose, isomaltose, maltulose, sucrose [8].

Syneresis is used to describe the separation of whey on the surface of the product due to the tightening of the gel structure created by fermentation, which is a common problem and deficiency in the production and storage of yogurt.

There is no standardized analytical procedure for measuring syneresis, so it is determined by different methods based either on the principle of gravity or on the principle of centrifugal force [9]. The causes of syneresis can be numerous, and the most common are inadequate parameters during the technological process of beverage production. The development of the technology of fermented milk drinks improves and expands the range of high-quality functional food. It is known that the type of starter culture determines the nutritional, rheological and sensory properties of the fermented milk product.

Table 1. Sample of fermented milk drink

Sample	% of non-traditional starter inoculum	Starter culture	Sweetener	% of kombucha fermented milk drink as inoculum	
				kb1	kb2
kb1	10	-	sucrose	-	-
kb2	10	-	honey	-	-
kb3	-	-	-	10	-
kb4	-	-	-	-	10
kb	-	Lyofast Y 452	-	-	-

kb-kombucha

EXPERIMENTAL

MATERIALS

Kombucha culture grown on black tea was used for the fermentation process. The tea was prepared by adding 8 g of tea to 2 L of water at a temperature of 95°C. After 5 minutes, the tea was filtered, cooled to room temperature and divided into 2 equal parts. 70 g/L of sugar (sucrose) was added to one part, and 61.25 g/L of meadow honey to the other part, and mixed well. In both parts, 10 vol % starter (fermented tea) and were added (Table 1). Fermentation was carried out in a dark place (7 days/25°C) until the formation of a new biofilm of the kombucha culture, which later separated. After the formation of a sufficient number of kombucha, the milk fermentation process was started.

UHT milk with 2.8% m.f. produced by "Meggle" was used for the production of the fermented milk drink. The milk was inoculated with 10% inoculum of non-traditional starter culture in relation to the total amount of milk (kb1 and kb2) and inoculated milk (kb3 and kb4) with 10% of kombucha drink

Fermented milk drink kb was produced using a lyophilized (FD-DVS, Frozen dried Direct Vat Set) culture (manufactured by Sacco Clerici, Italy) Lyofast Y 452 E (composed of: *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus*), while respecting all the culture manufacturer's parameters.

Fermentation of inoculated milk samples was carried out, with three times repetition, in a water bath at 34°C, with monitoring of the pH value up to 4.6. Then the samples were cooled in ice water, packed in sterile glass packaging and stored at a temperature of +4°C.

METHODS

DETERMINING PH VALUE

Active acidity was determined potentiometrically using a Testo 206 pH meter after the 1st, 7th, and 14th day of storage.

DETERMINING TITRATABLE ACIDITY

Titratable acidity was determined by Soxhlet-Henkel (°RH) after the 1st, 7th, and 14th day of storage.

DETERMINING SYNERESIS INTENSITY

Syneresis intensity was determined according to the modified method of Koegh and O'Keneedy [10] using a Tehtnica Železniki PLC 322 centrifuge. Samples of the fermented milk drink were centrifuged at 3000 rpm [11], and the volume of separated whey was expressed as ml of centrifuged/100 g of a sample [12].

STATISTICAL ANALYSIS

Statistical analysis (ANOVA) was performed using SPSS software (version 25), and Dunnet's test was used to assess statistical significance (p<0.05).

RESULTS AND DISCUSSION

Changes in the active and titratable acidity of the produced samples depending on the type of starter and sweetener are shown in Table 2. Table 3 shows results of syneresis intensity of the samples during 14 days storage.

During the storage of fermented dairy products, subsequent acidification of the curd occurs. Subsequent acidification is a consequence of the persistent metabolic activity of the product's microflora during its shelf life [13] and affects the appearance of whey syneresis [14]. The release of whey is considered one of the most important parameters of the quality of fermented dairy products because it indicates certain errors in the production process.

In all samples of fermented milk drink, the pH value decreased linearly. During the first day of storage, the control sample (kb) and sample kb2, where honey was used as a sweetener, had the lowest pH value. According to the results, the samples in which honey was used as a sweetener had a lower pH value compared to the samples in which sucrose was used.

The reason for this may be the lower pH value of honey, which ranges from 3.2 to 4.5 depending on the type of honey [15].

According to literature data [16, 17], meadow honey has a pH above 4. In samples where kombucha was used as a starter, the drop in pH value was not statistically significant compared to the control sample ($p>0.05$), for all storage days. During storage, there was an increase in titratable acidity in all samples, which indicates the subsequent activity of the starter and the production of lactic acid in the produced samples. Analysis of variance showed that there is a statistically significant difference in titratable acidity for all days of storage.

Dunnett's test showed that there was a statistically significant difference between kombucha and control samples after the 1st, 7th and 14th day of storage, except for kb3 and kb (on the 1st day of storage) and kb3 and kb (on the 7th days of storage). Similar results were obtained by Makvandi et al. [6] in their study, who used Kombucha extract in different %(v/v).

Table 2. Average values and standard deviation of acidity of fermented milk drink during 14 days storage

Samples	pH			Acidity (°SH)		
	1 day	7 day	14 day	1 day	7 day	14 day
kb	4.41±0.05	4.23±0.14	4.11±0.14	29.60±0.1	31.70±0.17	33.60±0.05
kb1	4.48±0.02	4.31±0.01	4.18±0.01	30.80*±0.17	32.93*±0.05	34.50*±0.23
kb2	4.41±0.04	4.28±0.25	4.09±0.11	33.00*±0.08	34.63*±0.05	36.30*±0.1
kb3	4.48±0.03	4.35±0.07	4.2±0.20	30.16*±0.05	31.85±0.13	33.20*±0.00
kb4	4.45±0.13	4.23±0.09	4.18±0.02	29.80±0.13	32.13*±0.05	33.10*±0.00

kb-kombucha

**- the mean difference is significant at the 0,05 level*

Table 3. Average values and standard deviation of syneresis intensity of fermented milk drink during 14 days storage

Sample	Intensity of syneresis		
	1 day	7 day	14 day
kb	11.33±0.11	12.33±0.05	13.53±0.05
kb1	11.20±0.26	12.40±0.34	13.65±0.27
kb2	10.11*±0.07	11.35*±0.13	11.98*±0.12
kb3	8.90*±0.27	10.20*±0.18	11.95*±0.26
kb4	7.14*±0.09	8.25*±0.08	9.38*±0.04

kb-komucha

**- the mean difference is significant at the 0,05 level*

Looking at the results in Table 3, all samples showed an increase in syneresis during storage, analogous to a decrease in pH value and an increase in titratable acidity [18, 19] because proteins lose their ability to bind to water when pH is applied. and increased acidity which also contributes to syneresis.
Analysis of variance showed that there is a statistically significant difference in syneresis intensity for all days of storage, and Dunnett's test showed that there is a statistically significant difference between kombucha samples and control samples during storage. During storage, the sample kb4 had the lowest syneresis intensity. For the first day of storage, the sample kb had the highest intensity of syneresis, and after day 7 and 14, it was sample kb1. Based on the results, it can be concluded that the use of kombucha and honey is the best combination that contributes to the stability of the drink's structure and indicates a better product quality. Stijepic et al. [11] in their research showed a positive effect of adding honey to yogurt on viscosity and water retention capacity, i.e. on reducing the intensity of syneresis. Samples that had sucrose as a sweetener also showed acceptable values of syneresis intensity. Sweeteners, such as sucrose, high-fructose

corn syrup, or honey, are commonly added to blended yogurts to mask acidity for acidity-conscious consumers and, perhaps, to produce a firmer texture [20]. This is a very important fact considering that sucrose and honey were used as sweeteners here.

CONCLUSION

In all samples, pH value decreased linearly during storage. The addition of honey affected the lower pH value of the samples. During storage, there was an increase in titratable acidity in all samples, due to the still active starter and further formation of lactic acid in the produced samples. Due to the decrease in pH value and increase in titratable acidity, there was an increase in the intensity of syneresis during storage in all samples, analogously to a decrease in pH value and an increase in titratable acidity. Kombucha as well as sweeteners contributed to the stability of the structure as well as the better quality of the produced samples.

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COMPARATIVE ANALYSIS OF THE ANTIOXIDANT CAPACITY OF SOME NATURAL AND SYNTHETIC ANTIOXIDANTS ADDED TO PALM OIL

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:
Various synthetic and natural antioxidants are used to reduce oxidation and its negative impact on the oil during the food frying process. Considering that some studies show the negative impact of synthetic antioxidants on the health of consumers, natural alternatives are being used more and more. BHA and BHT are synthetic antioxidants that are widely used in the food industry and a large number of natural compounds such as phenols, anthocyanins, flavonoids, vitamins, etc. show antioxidant properties. In this study, the antioxidant capacity of oregano and rosemary essential oil was tested and compared with the antioxidant capacity of BHA and BHT. The antioxidant capacity was evaluated by the removal of DPPH radicals and by iron reduction (FRAP). The results of this study showed that the studied essential oils exhibited antioxidant capacity. These oils have a high antioxidant capacity, however, compared to synthetic antioxidants, they show a significantly lower antioxidant capacity but they can be used as natural antioxidants during food processing.

KEYWORDS: antioxidant capacity; essential oils; synthetic antioxidants; lipid oxidation

INTRODUCTION

Lipid oxidation and oxidative stress occur due to various influences in the processes of food production, processing and preparation. This is recognized as a major problem in the use of edible oils during frying, as well as other methods of food preparation. Oxidation as well as oxidative stress cause negative changes in the chemical, sensory and nutritional properties of food.Various synthetic and natural antioxidants are used to reduce oxidation and its negative impact on the oil during the food frying process. Synthetic antioxidants are cheaper than natural ones, but it is generally accepted that natural antioxidants have a stronger, more efficient and health-safe effect than synthetic ones.

Antioxidants are chemical compounds that can be used to improve the oxidative stability of oils and fats by interrupting the free-radical mechanism or autooxidation [1].A large number of compounds of plant origin have antioxidant properties and can neutralize free radicals. Such compounds are most often phenols, carotenoids, anthocyanins, flavonoids, unsaturated fatty acids, vitamins, enzymes, etc. This has stimulated interest in using them as oxidants during food processing [2].

Antioxidants are compounds capable of donating hydrogen radicals to free radicals available to prevent oxidative damage [3]. Free radicals are highly reactive molecules with unpaired electrons that can cause various oxidative stresses. Oxidative stress involves the generation of reactive oxygen and nitrogen species. Such species have been implicated in aging and various pathological processes because they damage the structures of cells, lipids, membranes, proteins, and DNA [4].

People's eating habits are changing very quickly today, and fast food, usually fried, is often consumed. Although the common trend is to reduce or limit the consumption of fried foods, their consumption level is gradually increasing owing to their properties such as being tasty, easily prepared and micro-biologically safe. In order to minimize the negative effects and/or to maximize the positive health effects and to maintain the quality of the fried products it is necessary to addition of antioxidant additives into oil [1].

Lipid oxidation can be prevented by using various chemical additives. However, several questions about safety of these chemicals used for food preservation were raised. In fact, they were suspected to have negative effects on consumer's health. Thus, natural antioxidants have become highly demanded as alternatives and better choice to the chemical additives [5].Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used widely in the food industry to prevent lipid oxidation. BHA and BHT have been reported to have toxic and carcinogenic effects[6]. Therefore, natural antioxidants have attracted increased interest because natural ingredients may be safer than synthetic ingredients. As natural antioxidants, essential oils with high antioxidant capacity, such as oregano, rosemary, and other plant species rich in phenolic components, are increasingly being used. Essential oils are natural, volatile complex compounds characterized by the odor of their corresponding aromatic plants, which synthesize them as secondary metabolites [4].

In this paper focus is on oregano and rosemary essential oil. The compounds responsible for antioxidant activity of oregano include caffeic, coumaric and rosamarinic acids, carvacrol, thymol, and flavonoids. The compounds responsible for antioxidant activity of rosemary include phenolic acids (caffeic, ferulic, and rosmarinic acid) and phenolic diterpenes (carnosic acid and carnosol) [3]. The aim of this study is to compare the antioxidant capacity of natural antioxidants such as oregano and rosemary essential oil with synthetic antioxidants such as BHA and BHT, which are among the most commonly used in the food industry.

EXPERIMENTAL

MATERIALS

Natural and synthetic antioxidants were used in this research. As natural antioxidants were used oregano androsemary oil and as synthetic were used butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT).

Oregano oil (*Origanum vulgare*) - according to the declaration, it is 100% essential oil of wild oregano. It contains 85% carvacrol and 5% thymol. Country of origin is India.

Rosemary oil (*Rosmarinus officinalis, Lamiiaceae*) as cineol-type essential oil in a vegetable carrier, colorless to pale yellow in color, with a characteristic, refreshing scent of conifers was used. Rosemary oil is obtained by steam distillation from fresh rosemary flower tops. Country of origin is Bosnia and Herzegovina. According to the manufacturer's declaration, rosemary essential oil contains: 1,8-cineole, monoterpenes: alpha and beta pinene, camphene and limonene, alcohols, monoterpenols (linalool, alpha-terpineol and borneol), and iso-bornylacetate ester, and contains ketones: camphor, verbenone and thujone.

Butylated hydroxyanisole - (2-*tert*-butyl-4-methoxyphenol) – BHA, serial number: 1001176424, manufacturer: MERCK, Germany.

Butylated hydroxytoluene - (2,6-Di-*tert*-butyl-4-methylphenol) – BHT, serial number: 10112785, manufacturer: MERCK, Germany.

METHODS

PREPARATION OF SAMPLES

Extraction of active substances from the oil: Weigh about 1 g of oregano and rosemary oil into a 100 mL erlenmayer flask and add 9 mL of methanol. Samples were placed on a shaker for 1 hour (400 beats per minute). After that, the samples were transferred to cuvettes and centrifuged for 10 minutes at 3000 rpm. Then, the supernatant was transferred to a 10 mL flask and filled with methanol to the mark. For oregano oil, a dilution of 1000 was taken.

Synthetic antioxidants: Weighed 10 mg in a 10 mL flask, dissolved in methanol and filled to the mark. Dilutions were made from this, 1 mL in 10 mL.

The antioxidant activity was determined spectrophotometrically on a UV-VIS spectrophotometer (Shimadzu UV-1800) using two methods: the DPPH method, which uses 2,2-diphenyl-1-picrylhydrazyl radical as the radical, and the FRAP method.

DPPH RADICAL SCAVENGING ACTIVITY ASSAY

The ability of the extract to act as a free synthetic DPPH radical scavenger was measured by the decrease in absorbance at a wavelength of 517 nm after the addition of the extract [7]. Determination of the antioxidant capacity was determined by the DPPH method, i.e. neutralization of the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical according to the method described in the work by Matejić et al. (2013) with minimal changes [8].

A specified sample volume (10-100 μL) is taken, add methanol to 4 mL and 1 mL of 0.5 mM DPPH solution in methanol. After 30 minutes of reaction at room temperature in the dark, the absorbance value was measured at wavelenght 517 nm using a UV-VIS spectrophotometer (Shimadzu UV-1800) against a blank. The blank composed of 4 mL of methanol without the extract mixed with 1 mL of 0.5 mM DPPH solution in methanol. The radical scavenging activity of each solution was calculated as the inhibition percentage according to the following equation:

$$\% \text{ Inhibition} = [(A_{blank}-A_{sample})/A_{blank}]\times 100 \qquad (1)$$

where A_{blank}: the blank absorbance at 517 nm, A_{sample}: the sample absorbance at 517 nm. The percentage inhibition of DPPH was expressed as IC50. The IC50 value defined as the concentration of antioxidant that could reduce the initial concentration of free radicals to 50%[9].

FERRIC ION REDUCING ANTIOXIDANT POWER (FRAP) ASSAY

The FRAP method is based on the ability of the extract to reduce F^{3+} ions to Fe^{2+} ions. The resulting Fe^{2+} ions with the TPTZ reagent (2,4,6-tri(2-pyridyl)-s-triazine) form a blue-colored complex, which reaches an absorption maximum at 593 nm [10].

The FRAP reagent was prepared by mixing 25 mL of 0.3 M acetate buffer (pH 3.6), 2.5 mL TPTZ (10 mM, in 40 mM HCl) and 2.5 mL $FeSO_4 \times 6H_2O$ (20 mM). The FRAP reagent is thermostated at 37°C. All solutions were prepared on the day of use. 3 mL of FRAP reagent freshly was added to 100 µL of the extract and incubated in a water bath for 30 minutes at 37 °C. After 30 minutes, the absorbance value was measured at wavelenght 593 nm using a UV-VIS spectrophotometer (Shimadzu UV-1800) against a blank. The blank composed of 3 mL of FRAP solution mixed with 100 µL of methanol.

Series of standard $FeSO_4 \times 7H_2O$ solutions in concentrations of 200-1000 µmol/L was prepared to create a standard curve. FRAP values were calculated according to the calibration curve for $FeSO_4 \times 7H_2O$:

$y=0.0008x+0.0681$

where y is the absorbance at 593 nm and x is the concentration of $FeSO_4 \times 7H_2O$ in µmol/L; $R^2=0.9952$. FRAP values were expressed as µmol Fe^{2+}/g of sample.

RESULTS AND DISCUSSION

The results of the antioxidant capacity estimated by DPPH and FRAP assay are shown in Table 1. As stated in the experimental part, the percentage of DPPH radical inhibition is expressed as IC50. The IC50 value is defined as the concentration of antioxidants that can reduce the initial concentration of free radicals to 50%. The lower the IC50 value, the greater the antioxidant capacity of that sample, i.e. a lower concentration of antioxidants is needed to inhibit the DPPH radical.

Table 1. Antioxidant activity of BHA, BHT, oregano and rosemary oil determined by DPPH and FRAP assay.

Sample	IC50 (mg/mL)	FRAP(µmol Fe^{2+}/1 g of sample)
BHA	0.0052	12341
BHT	0.011	9928
Oregano oil	0.474	4304
Rosemary oil	56.61	3584

The IC50 value for synthetic antioxidants (BHA and BHT) is 0.0052 and 0.011mg/mL respectively. According to Ceylan et al. [11], the IC50 value for BHAis 0.035± 0.007mg/mL and for BHT0.020± 0.001mg/mL. The FRAP value for BHA is 12341µmol Fe^{2+}/g of sample and BHT 9928µmol Fe^{2+}/g of sample. According to Ceylan et al., the FRAP value is higher for BHA than for BHT, and according to Kasote et al. [12] it is the other way around, although the values are approximately the same. The FRAP value for BHA is 8333± 7.44, and for BHT 8666± 7.22µmol Fe^{2+}/g of sample according to Kasote et al. According to the results in this work, the antioxidant capacity is higher for BHA.

When it comes to natural antioxidants, it can be noted that oregano oil has a significantly better IC50 value than rosemary oil, while the results for the FRAP value don't show such a difference. The IC50 value for natural antioxidants (oregano and rosemary oil) is 0.474mg/mL and 56.61 mg/mL, respectively. The FRAP value for oregano oil is 4304µmol Fe^{2+}/1 g, and for rosemary 3584 µmol Fe^{2+}/1 g of sample. Figure 2 shows the results of FRAP values graphically. Wang et al. [13] investigated the antioxidant capacity of rosemary essential oil from China and the Mediterranean region, and obtained IC50 values of 21.56mg/mL, 23.8mg/mL and 16.45 mg/mL.They also determined the antioxidant capacity using other methods, and came to the conclusion that the antioxidant activity of rosemary showed that samples grown in China perform better than those of the Mediterranean region [13]. It can be concluded that the concentration of essential oil compounds varies depending on the time of harvest, growing conditions, geographical location, altitude, climate, genetic diversity and phenological age of the plant at the time of cutting [14]. According to Almeida et al. [15]the IC50 value for oregano oil is 0.5 mg/mL, which is almost the same value as in this study.

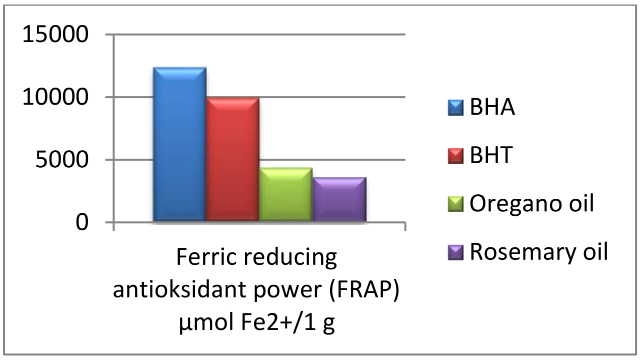

Figure 1. Graphically representation of FRAP values.

From the presented results, it can be seen that synthetic antioxidants have a higher antioxidant capacity than natural ones. Butylated hydroxyanisole (BHA, E320) has the highest antioxidative capacity, followed by butylated hydroxytoluene (BHT, E321), oregano essential oil, and finally rosemary essential oil. The antioxidant capacity values determined by both methods were generally parallel to each other.

This following fact should also be taken into account that BHA and BHT are very effective during the storage and transport of oils and fats, but they are less effective at frying temperatures, due to their volatility. Also, rosemary extracts are particularly active as antioxidants at the high temperatures in frying fats. They protect the oils during frying and their antioxidant activity is carried over into the fried foods[6].

CONCLUSION

In recent years, studies have focused on natural antioxidants such as essential oils because of the toxicity and carcinogenicity of synthetic antioxidants. The results of this study showed that the studied essential oils exhibited antioxidant capacity. These oils have a high antioxidant capacity, however, compared to synthetic antioxidants, they show a significantly lower antioxidant capacity. However, looking at the long term and taking into account the health aspect and food safety, it is better to use natural ones.According to these results, essential oils can be used as natural antioxidants during food processing, to prevent lipid oxidation. Especially when compared to synthetic antioxidants, natural antioxidants are easily acceptable by consumers. They are considered safe, the legislation does not require any safety tests. People have used such additives for hundreds of years before and they not only prolong the shelf life, but also increase the nutritional value of foods.

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POSSIBILITY OF BLEACHING SUNFLOWER OIL WITH SYNTHETIC ZEOLITE

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:
In this paper, the possibilities and effects of using domestic industrially produced zeolite for bleaching crude sunflower oil, compared to imported commercial bleaching earth, were examined. The parameters of the bleaching process in laboratory conditions were: temperature 95°C, contact time 30 min, and mass fractions of bleaching agent in oil: 0.2, 1, 2 and 3%. The following methods were used to characterize the bleaching agents: XRD, FTIR, BET and SEM/EDS. The bleaching efficiency parameters were determined by laboratory methods prescribed by the relevant ordinance on edible vegetable oils, and included: soap content, peroxide value and fatty acid composition. The results of the research showed that the effects of bleaching sunflower oil with synthetic zeolite are similar to the effects of bleaching with imported commercial bleaching earth, with the former showing a slightly higher effectiveness in reducing the peroxide value.

KEYWORDS: sunflower oil; oil bleaching; synthetic zeolite; bleaching earth

INTRODUCTION

Crude vegetable oil contains undesirable substances such as free fatty acids, gums and dyes, soap residues, phosphatides and traces of metals, and these substances affect the efficiency of its processing process, as well as the quality and market value of the final product [1]. Therefore, in the industrial production of edible vegetable oils, the bleaching procedure of previously neutralized crude oil plays a major role, in which unwanted substances are removed by applying a suitable adsorbent [2], which enables the production of commercial oil of the quality prescribed by relevant national regulations. Natural and active clays are usually used as bleaching agents for edible vegetable oils [3], [4], and clay activation is usually done with inorganic acids such as H_2SO_4 [5], [6] and HCl [7], [8]. The goal of activation is to improve the physical and chemical properties responsible for the removal of unwanted substances from oil [9], and refers, among other things, to the increase of adsorption capacity [10], specific surface area and microporosity of clay [11], [12], [13]. The optimal activation process conditions are specific for each clay [14], which is why different materials are continuously tested in laboratory and semi-industrial conditions.

Producers of edible vegetable oils bleach crude oils under different process conditions (temperature, time, dose of bleaching agent), which mostly depend on the type of oil, type of bleaching agent and equipment used. At the same time, researchers are continuously investigating possibilities of using different natural, synthetic, or waste materials, either unactivated or previously activated, as bleaching agents in order to obtain the required oil quality with greater economic profitability and environmental protection [15], [16], [17], [18].

Among the available adsorbents, zeolites are considered to be inexpensive materials [19]. Namely, although zeolites are available in nature, their industrial synthesis is relatively easy and enables the product to be obtained in a pure form, so it is most commonly practiced [20]. In this paper, the characteristics of the domestic synthetic zeolite and the possibility of its application in the bleaching process of raw sunflower oil were examined, in comparison with imported commercial bleaching earth.

MATERIALS AND METHODS

The following materials were used in the experimental part of the research: synthetic zeolite ZEOflair 100 (Zeochem, Zvornik), imported commercial bleaching earth and raw sunflower oil (Bimal, Brčko), and other reagents and chemicals required for the characterization of bleaching agents. Bleaching of sunflower oil was performed by adding a certain amount of bleaching agent to glasses with 200 ml of raw sunflower oil preheated to 95°C, and then intensively mixing the suspension for 30 minutes. The applied mass fractions of bleaching agents in oil were (*w*): 0.2, 1, 2 and 3%. At the end of the mixing time, the suspension of oil and bleaching agent was filtered on vacuum filtration equipment.

The following methods were used to characterize the synthetic zeolite and commercial bleaching earth: X-ray diffractometry (XRD), infrared spectroscopy (FTIR), low-temperature nitrogen adsorption (BET), and scanning electron microscopy with energy dispersive spectrometry (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 the range of wavelengths 4000 - 500 cm^{-1}.

Low-temperature nitrogen adsorption was performed on a Micrometrics ASAP 2010 instrument, which determined the textural characteristics of synthetic zeolite and commercial bleaching earth.

The morphological characteristics of the bleaching agents were determined with an electron microscope JEOL-JSM-6460LV at a resolution of 3-4 nm and a magnification of 500-3000 times. 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. Elemental microanalysis was performed with an energy dispersive spectrometer with analyzer, Noran System Six 200 (detection of elements $Z \geq 5$, detection limit of mass fraction ~ 0.1%, resolution 126 eV).

To characterize sunflower oil before and after bleaching, as well as to determine the effects of bleaching, standardized methods prescribed by the Rulebook on the Quality of Edible Oils, Fats and Mayonnaise in Bosnia and Herzegovina [21] were used, which determined the peroxide value and soap content.

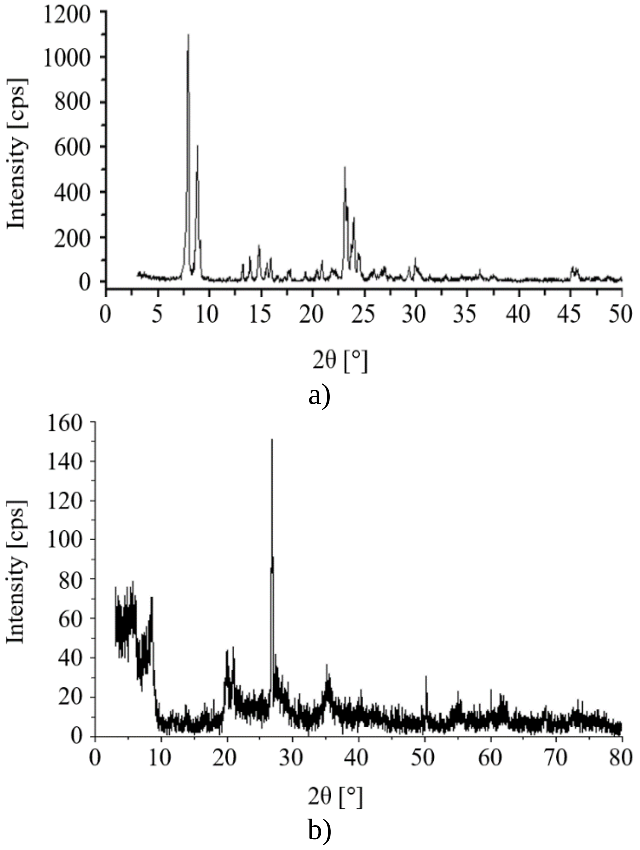
The fatty acid composition of the oil was determined by gas chromatography (GC-FID) on an Agilent 7890 GC gas chromatograph. The retention time for individual fatty acids was determined from the obtained chromatograms, and then their mass fractions in the oil samples were determined based on the area and height of the fatty acid peaks.

RESULTS AND DISCUSSION

RESULTS OF CHARACTERIZATION OF SYNTHETIC ZEOLITE AND COMMERCIAL BLEACHING EARTH

Figure 1 shows the diffractograms of synthetic zeolite samples and commercial bleaching earth. Based on the obtained values of intensity, *I* (cps) and interplane distances *d* (Å), and by comparing with the interplane distances, it was determined that the examined zeolite sample has a crystal structure of ZSM-5 (MFI) zeolite.

In the diffractogram of a sample of commercial bleaching earth, in addition to smectite and quartz peaks, peaks of phyllosilicate minerals were also identified, such as palygorskite.



a)

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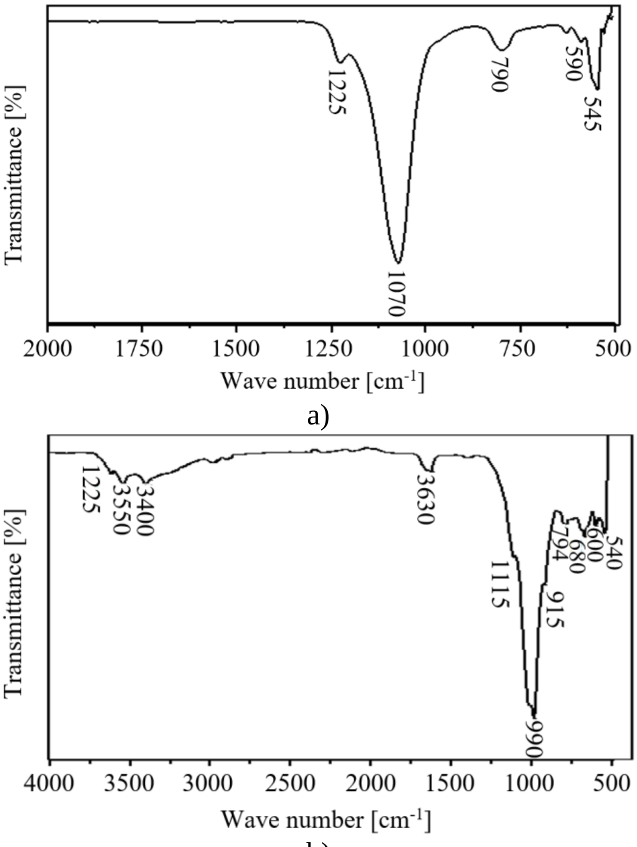
Figure 1. Diffractograms of: a) synthetic zeolite; b) commercial bleaching earth

Figure 2 shows the FTIR spectra of synthetic zeolite and commercial bleaching earth. The FTIR spectrum of the synthetic zeolite shows bands characteristic of ZSM-5 type zeolite. 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 FTIR spectrum of commercial bleaching earth shows bands characteristic of aluminosilicate minerals. The band at ~3600 cm^{-1} originates from stretching vibrations of OH groups coordinated by octahedral Al^{3+}, while the bands at 3400-1630 cm^{-1} originate from stretching and bending OH vibrations of water. The bands in the region of 1020-990 cm^{-1} originate from Si-O and Si-O-Si stretching vibrations, while the band at ~915 cm^{-1} originates from Al-Al-OH bending vibrations. The bands at ~680 cm^{-1} and ~540 cm^{-1} originate from Si-O and Al-O-Si vibrations. A broad band in the range ~790 cm^{-1} to ~750 cm^{-1} indicates the presence of quartz.



a)

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Figure 2. FTIR spectra of: a) synthetic zeolite; b) commercial bleaching earth

Figure 3 shows the adsorption isotherms of synetic zeolite and commercial bleaching earth, and Table 1 shows some of their textural characteristics. The zeolite sample is characterized by a Type I adsorption isotherm for microporous materials [22], where based on the appearance of the isotherm itself and the high value of the specific surface area (325.90 m^2/g), it can be concluded that the sample is dominated by micropores with a certain proportion of small mesopores. Commercial bleaching earth is characterized by a type II adsorption isotherm, which is typical for nonporous or macroporous materials [23].

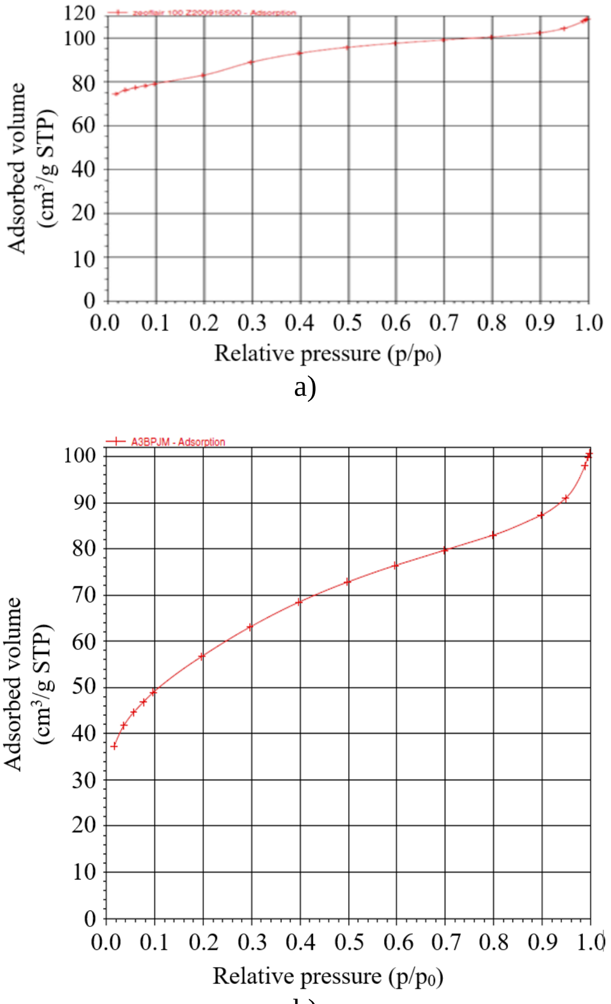

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Figure 3. Adsorption isotherms of: a) synthetic zeolite; b) commercial bleaching earth

The results in Table 1 show that, compared to the commercial bleaching earth, the synthetic zeolite has higher values of specific surface area (SP_{BET}) by 1.91 times, micropore area ($S\mu p$) by 4.96 times, and micropore volume ($V\mu p$) by 5.35 times. However, commercial bleaching earth has mean pore diameter (*dp*) and specific external surface area (*SPext*) values 3.11 and 1.09 times higher.

Figures 4 and 5 show SEM micrographs of synthetic zeolite and commercial bleaching earth, at magnifications of 500 and 3000 times. Synthetic zeolite particles are smaller than those of commercial bleaching earth and have significantly higher microporosity, which is confirmed by the fact that the mean pore diameter of synthetic zeolite is smaller than the mean pore diameter of commercial bleaching earth by 3.11 times.

Table 1. Textural characteristics of synthetic zeolite (SZ) and commercial bleaching earth (ZB)

Characteristic	Bleaching agent	
	SZ	**ZB**
Specific surface area (*SP_{BET}*), m^2/g	325.90	170.6072
Specific external surface area (*SPext*), m^2/g	113.19	122.9679
Constant (*C_{BET}*)	-66.3216	357.5192
Micropore surface (*$S\mu p$*), m^2/g	212.702	47.639
Micropore volume (*$V\mu p$*), cm^3/g	0.111246	0.020798
Mean pore diameter (*dp*), nm	3.8359	11.9390

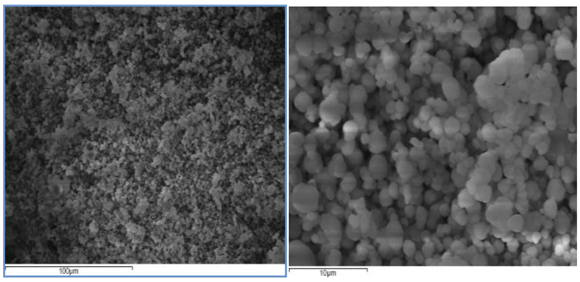
a) b)

Figure 4. SEM micrographs of synthetic zeolite at magnifications of: a) 500 times; b) 3000 times

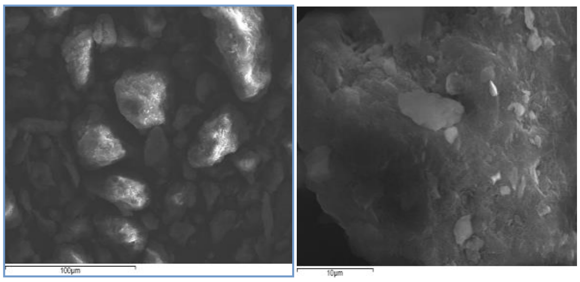
a) b)

Figure 5. SEM micrographs of commercial bleaching earth at magnifications: a) 500 times: b) 3000 times

Table 2 shows the mass fractions (*w*) and atomic percentages (*at.*) of individual elements in synthetic zeolite and commercial bleaching earth, obtained by elemental microanalysis (EDS). The zeolite sample has a regular spherical shape and represents silicalite, in which the presence of aluminum and sodium was not detected, and it has a modulus SiO_2/Al_2O_3 >500 (∞). The oxide composition of commercial bleaching earth (*w*) is: 88.32% SiO_2, 7.43% CaO, 1.40% K_2O and 2.85% Fe_2O_3.

Table 2. Elemental analysis of synthetic zeolite (SZ) and commercial bleaching earth (ZB)

Element	SZ, (*w*) %	SZ, (*at*) %	ZB, (*w*) %	ZB, (*at*) %
O	60.03	72.50	68.89	80.93
Al	0.00	0.00	-	-
Si	39.97	27.50	24.46	16.37
Fe	0.00	0.00	3.16	1.06
Ti	0.00	0.00	-	-
K	-	-	0.54	0.26
Ca	-	-	2.94	1.38

THE RESULTS OF SUNFLOWER OIL CHARACTERIZATION BEFORE AND AFTER BLEACHING

The quality of edible vegetable oils in Bosnia and Herzegovina is defined by the relevant Rulebook [21] which prescribes that refined vegetable oils must meet the following requirements: that at a temperature of 25°C they are clear, have a mild and pleasant taste and smell, the color characteristic of the raw material, the mass fraction of free fatty acids (expressed as oleic acid) maximum 0.3%, mass fraction of water and other volatile substances maximum 0.2%, soap content maximum 50 mg/kg (as Na-oleinate) and peroxide value maximum 7 mmol O_2 per kg of oil. The effects of bleaching crude sunflower oil with synthetic zeolite and commercial bleaching earth were determined by methods for determining soap content, peroxide value and fatty acid composition.

The results of soap determination in sunflower oil samples after bleaching with synthetic zeolite and commercial bleaching earth showed that there are no residual soaps in them, i.e. their content was 0.00 mg/kg.

Peroxide value (PV) is a measure of oxidation and change in oil quality (mmol/kg). The determined peroxide value of raw sunflower oil was 6.53 mmol/kg. Table 3 shows the peroxide values of sunflower oil after its bleaching with different mass fractions (*w*) of synthetic zeolite and commercial bleaching earth in oil. The peroxide values of all sunflower oil samples after bleaching with synthetic zeolite and commercial bleaching earth are in accordance with the national regulation on the quality of edible vegetable oils [21]. The results show that increasing the dose of bleaching agent led to a decrease in the peroxide value of the oil, with the synthetic zeolite showing higher efficacy than the commercial bleaching earth. However, a visual comparison of the color of sunflower oil samples after bleaching, with the color of crude and commercial refined oil, showed slightly better effects of changing the color and clarity of the oil using commercial bleaching earth. The reasons for the insufficient change in color and clarity of the oil can be the age of the sunflower oil sample, possibly inadequate filtering equipment, as well as the failure to add activated carbon to the specified bleaching agents.

Table 3. Peroxide values of sunflower oil after bleaching with synthetic zeolite (SZ) and commercial bleaching earth (ZB)

SZ		ZB	
(*w*) %	**(*PV*) mmol/kg**	**(*w*) %**	**(*PV*) mmol/kg**
0.2	4.365	0.2	5.75
1.0	3.775	1.0	4.377
2.0	2.475	2.0	3.316
3.0	1.495	3.0	1.229

The types and mass fractions of fatty acids that make up the vegetable oil depend on its type. In tables 4-6. the results of the analysis of the fatty acid composition of sunflower oil samples before and after bleaching with synthetic zeolite and commercial bleaching earth are given. Crude sunflower oil contains the following fatty acids: linoleic, oleic, pamitic, stearic and behenic. In this sample, the mass fractions of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) are (*w*): 10.61%, 29.62% and 59.12%. Due to the high content of essential fatty acid (linoleic acid), sunflower oil belongs to the linoleic type of oil [24] and is considered a high-quality edible oil [25],]26].

Table 4. Fatty acid composition of the crude sunflower oil sample

Fatty acid (symbol)	**(*w*) %**
Palmitic (C16:0)	6.43577
Stearic (C18:0)	3.48085
Oleic (C18:1)	29.62076
Linoleic (C18:2)	59.1215
Behenic (C22:0)	0.69442

Table 5. Fatty acid composition of sunflower oil samples after bleaching by synthetic zeolite

Dose of synthetic zeolite ,(*w*) %	**Fatty acid mass fraction, (*w*) %**				
	Palmitic	**Stearic**	**Oleic**	**Linoleic**	**Behenic**
0.2	6.72264	3.57067	28.78736	60.20781	0.71153
1.0	6.68726	3.57808	28.91326	60.1052	0.7162
2.0	6.46943	3.49522	29.57257	59.06908	0.73746
3.0	6.6144	3.56085	29.0606	60.02001	0.74414

Table 6. Fatty acid composition of sunflower oil samples after bleaching with commercial bleaching earth

Dose of commercial bleaching earth, (*w*) %	**Fatty acid mass fraction, (*w*) %**				
	Palmitic	**Stearic**	**Oleic**	**Linoleic**	**Behenic**
0.2	6.60466	3.5589	29.03599	60.10005	0.7004
1.0	6.70676	3.57828	29.04598	59.97751	0.69147
2.0	6.67418	3.57208	29.05314	60.00552	0.69508
3.0	6.61437	3.56525	29.07031	60.06984	0.68023

The results of the analysis of sunflower oil after bleaching with synthetic zeolite and commercial bleaching earth show that there was a slight change in the fatty acid composition, namely: an increase in the content of palmitic, stearic, linoleic and behenic acids, and a decrease in the content of oleic acid. Consequently, there was a slight change in the proportion of fatty acids, i.e. the proportion of SFA and PUFA increased, and the proportion of MUFA decreased.

CONCLUSIONS

In this paper, the possibility of using domestic synthetic zeolite ZEOflair 100 for bleaching sunflower oil, as a possible substitute for imported commercial bleaching earth, was examined. The effects of bleaching sunflower oil with synthetic zeolite were compared with the effects of bleaching with imported commercial bleaching earth. The characterization of synthetic zeolite and commercial bleaching earth was performed using modern test methods, such as: X-ray diffractometry, infrared spectroscopy, low-temperature nitrogen adsorption and scanning electron microscopy with energy dispersive spectrometry. Using the methods of X-ray diffractometry and infrared spectroscopy, it was determined that the examined zeolite sample has a crystal structure of ZSM-5 zeolite, while the commercial bleaching earth consists of smectite and quartz, and phyllosilicate minerals. Using the BET method, it was determined that the sample of synthetic zeolite, as a microporous material, is characterized by adsorption isotherm type I, and commercial bleaching earth by adsorption isotherm type II, which is typical for non-porous or macroporous materials. Based on the results of the textural characteristics, it can be concluded that the synthetic zeolite has values of specific surface, micropore surface and micropore volume that are 1.91 times, 4.96 times and 5.35 times higher than those of commercial bleaching earth. Commercial bleaching earth has higher values of mean pore diameter and external specific surface, by 3.11 times and 1.09 times.

Using the SEM/EDS method, it was determined that the synthetic zeolite is of a regular spherical shape and represents silicalite, in which the presence of aluminum and sodium was not detected, and it has a modulus of SiO_2/Al_2O_3 >500 (∞). The oxide composition of commercial bleaching earth (*w*) is: 88.32% SiO_2, 7.43% CaO, 1.40% K_2O and 2.85% Fe_2O_3.

The quality of edible vegetable oils in Bosnia and Herzegovina is defined by the relevant regulation, and the effects of bleaching raw sunflower oil with synthetic zeolite and commercial bleaching earth are determined by methods for determining its soap content, peroxide value and fatty acid composition. The analysis of sunflower oil samples before and after bleaching found that there are no residual soaps in them, while the peroxide values are significantly lower than those prescribed by the national regulations. By increasing the proportion of bleaching agents, there is a greater decrease in the peroxide value. The fatty acid composition of sunflower oil before and after bleaching with different mass fractions (0.2%, 1.0%, 2.0% and 3.0 %) of individual bleaching agents was determined by the gas chromatography method. The results showed that sunflower oil before bleaching contains: saturated fatty acids (palmitic, stearic and behenic), monounsaturated fatty acids (oleic) and polyunsaturated fatty acids (palmitic). After bleaching the oil with synthetic zeolite and commercial bleaching earth, there was a slight change in the proportion of saturated, monounsaturated and polyunsaturated fatty acids. The bleaching process increased the proportion of saturated and polyunsaturated fatty acids, while the proportion of monounsaturated fatty acids decreased.

The effects of using the researched bleaching agents are similar, with a slightly higher efficiency of synthetic zeolite in reducing the peroxide value of oil. In order to obtain better results and possibly carry out semi-industrial research, laboratory-scale research should be continued using more adequate laboratory equipment and packaging for oil samples, faster analyzes of oil samples after bleaching, as well as using other types of zeolites, combinations of zeolites with different porosity, or combinations of zeolites and activated carbon.

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EGC 2023 CONFERENCE ANNOUNCEMENT

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1ST EUROPEAN GREEN CONFERENCE – EGC 2023

Motivated by the importance and necessity of applying multidisciplinary and connecting all scientific disciplines in the field of environmental protection, the International Association of Scientists and Experts for Environmental Protection (IAESP) and the Faculty of Food Technology Osijek with the support of partner institutions, organizes the **1st European GREEN Conference**, which will be held from **23 to 26 May 2023** in **Vodice, Croatia.**

The main goal of the 1st European GREEN conference is to gather scientists and experts from all European countries and beyond in one place, and contribute to their connectivity and knowledge exchange in order to emphasize the necessity of applying a multidisciplinary approach in the field of environmental protection and sustainable development.

We are inviting scientists and professionals from all scientific fields to join us in EGC 2023, and through the oral or poster presentations contribute to sustainability, mitigating the climate crisis, and fulfillment of goals of the UN’s Sustainable Development Goals (SDG) and goals of the European Green Deal.

We also invite companies and consultants to participate in EGC 2023 and use the opportunity to promote and present their products and services related to environmental monitoring and protection, implementation, and supervision of green technologies through sponsored lectures, exhibitions, or demonstrations.

The EGC 2023 is organized by the International Association of Environmental Scientists and Professionals (IAESP), the Faculty of Food Technology Osijek, and a network of partner institutions.

EGC 2023 THEMATIC AREAS:

https://iaesp.org/egc-tematic_areas/

IMPORTANT DATES

15 March 2023 - abstract submission
15 May 2023 - registration of participants and fee payment
15 July 2023 - full paper submission

ABSTRACT AND PAPER SUBMISSIONS

The abstracts of the papers will be published in the Book of Abstracts of the 1st European GREEN Conference. Papers will be published in the Proceedings or in the supporting journal. All submitted abstracts and papers will undergo an international peer review process.

SUPPORTING JOURNALS:

Sustainability - Special Issue "A Multidisciplinary Approach to Sustainability" edited by the Guest Editor Prof. Dr. Mirna Habuda-Stanić. Advances in Civil and Architectural Engineering, Chemistry in Industry – KUI, Croatian Journal of Food Science and Technology, Ekonomski vjesnik – Econviews, Journal of Health Sciences, Life and School, Pravni Vjesnik, Southeastern European Medical Journal, The Holistic Approach to Environment, Technologica Acta, Tehnički Vjesnik - Technical Gazette.

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INSTRUCTION FOR AUTHORS

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