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INFLUENCE OF PROCESSING TECHNOLOGY ON BIOACTIVE COMPONENTS OF SOUR CHERRY

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

Cherry (*Prunus cerasus var. Oblačinska*) is one of the most common varieties in Bosnia and Herzegovina and the region. Owing to the excellent sensory and physico-chemical characteristics and the very high concentration of biologically active ingredients, it is a very good potential for obtaining value added products or products with high content of bioactive, nutritionally and biologically valuable ingredients. The processing techniques of fresh fruit have a large influence on the content of bioactive components.

The aim of this study was to analyse the effects of processing technologies of sour cherries on the content of bioactive compounds (total phenols, total flavonoids and anthocyanins) and the antioxidant capacity. Products that were analysed were juice, concentrate, extra jam and lyophilisate. The content of bioactive components and antioxidant capacity of these products was compared with fresh cherries. The results showed that the greatest influence on the content of bioactive ingredients has process where vacuum concentration is applied. More sophisticated technology such as lyophilization would contribute to the retention of bioactive components and could increase product competitiveness at the market.

Keywords: sour cherries, processing, bioactive components, antioxidant capacity

INTRODUCTION

Owing to the fact that different types of fruit show a positive health effects, many studies were conducted to determine the properties of certain bioactive compounds and to confirm the correlation between the chemical composition and the favourable health effects[1]. Phytochemicals as secondary plant metabolites are biologically active compounds of plant. In the human body they have a protective effect towards various diseases, particularly cardiovascular diseases and cancer[2]. Phytochemicals include a large number of structurally different compounds: organosulfur compounds (glucosinolates and their breakdown products, allyl and analogues in the onion), polyphenolic compounds (phenolic acids, flavan-3-ols, flavanones, flavanolic glucosides), carotenoids, isoflavones, dietary fiber in fruits, vegetables and grains. Many phytochemicals exhibit antioxidant activity and contribute to protecting cells against oxidative damage[3]. Fruits are considered a natural source of antioxidants that include anthocyanins and polyphenols, which reduce the risk of diseases related to oxidative stress, such as cancer and cardiovascular diseases[4]. The steady increase in the market for functional foods that is rich in antioxidants has led to increased interest in natural sources of antioxidants and their potential utilization as a nutrient and functional food ingredients. Flavonoids are perhaps the most beneficial phytochemicals found in food. It has been shown that flavonoids have antibacterial, sedative, anti-allergic, antimutagenic, antiviral and other effects[5]. Studies have shown that flavonoids have an excellent antioxidative and antiradical activity and thus play an important role in the pharmaceutical and food industry as antioxidants[6]. More than 6400 flavonoids have been identified[7].

The protective role of flavonoids in biological systems is attributed to their multiple actions such as capacity to capture electrons of free radicals, the ability of chelating binding of transition metal ions (Fe^{2+}, Cu^{2+}, Zn^{2+} and Mg^{2+})[8], activation of antioxidant enzymes[9] and inhibition of oxidase[10]. The mechanism of action of flavonoids on the molecular level in biological systems is not completely understood, due to significant

differences in chemical properties and also because of their great structural heterogeneity of flavonoids.

Anthocyanins are water soluble pigments in plants. They structurally belong to flavonoids and are well known for their health effects[11].

Cherries are taxonomically classified in the genus Prunus, which is part of the Rosaceae family. Cherry is an industrial fruit species and it is a significant raw material for the production of high quality syrup, tart and jam. The fruit of cherry is juicy and due to the presence of anthocyanins is red in color. Cherry (Prunus cerasus var. Oblačinska) is one of the most common varieties in Bosnia and Herzegovina and the region. Owing to the excellent sensory and physico-chemical characteristics and very high concentration of biologically active ingredients, it is very good potential for obtaining value added products, or products with the high content of bioactive, nutritionally and biologically valuable ingredients. In order to avoid a loss of nutritional value of fruits, it is best to consume fresh fruit. However, it is often not possible, due to the need of transportation, storage, extending durability and the like. Therefore, the fruit should be processed. Since processing of fresh fruit have a large influence on the content of bioactive components, it is necessary to conduct studies to determine the effects of processing on the content of phytochemicals.

The aim of this study was to analyse the effects of different ways of processing of sour cherries, such as freeze drying, vacuum concentration and concentration on the content of bioactive compounds, particularly phenols, total flavonoids and anthocyanins. Also, the influence of processing on the antioxidant capacity of cherries was compared. The products that were analysed included lyophilisate (obtained by lyophilization process), dried sour cherry (obtained by vacuum drying) and concentrate (obtained by evaporation concentrating). The content of bioactive components and antioxidant capacity of these products was compared with fresh cherries and sour cherry jam.

MATERIALS AND METHODS

Cherry

Autochthonous sort of cherry “Oblačinska” was used. Fresh cherry, cherry juice, concentrate juice cherry, extra jam, jam and freeze-dried cherries were analysed and compared. Cherry juice was prepared by pressing and squeezing fresh cherries. The concentrate (juice concentrate) cherry was obtained on a laboratory BUCHI Rotavapour R-210 by evaporation of cherry juice at 50°C and pressure 75 mbar. Cherry extra jam was prepared by using sucrose and pectin as a gelling agent. For extra jam 60 g fruit/100 g jam was used. Pectin preparation was added in an amount of about 0,3-0,5% and the amount of sucrose and cooking time were set so that the final dry matter content of the product was at least 65% (Brix). The freeze-dried (lyophilisate) cherry was obtained by freeze drying in the lyophilizer Leybold-Heraeus GT2. Lyophilization was carried out at -40°C. The initial pressure was 2,5 mbar and after completion of lyophilization process it was 1,0 mbar. Duration of lyophilizing process was 47 hours.

Samples

For further analysis extract of fresh sour cherries, extra jam and dried cherry were prepared in triplicate. 100 g edible part of the fruit or jam was mixed in a blender to obtain a homogeneous sample. 10 g of the homogenized sample was extracted in an ultrasonic bath with 30 ml of methanol/HCl (95:5, v/v). After 30 min the solution was filtered and the residue on the filter paper was extracted again in the same way. The extracts were combined and diluted to 50 ml with a solution of methanol/HCl. Cherry juice and juice concentrate were analysed directly or diluted if necessary. Experiments were repeated at least three times. All results are given as mean ± standard deviation (SD).

Total phenolic content (TPC)

Total phenol content in the extracts was determined spectrophotometrically after reaction with Folin-Ciocalteu phenol reagent[12]. Samples were dissolved in methanol to a final concentration of 0,25 mg/ml. 50 ml of extracts, 450 ml deionized water and 2,5 ml of Folin-Ciocalteu reagent were mixed and incubated for 5 min. 2 ml of 7% sodium bicarbonate solution was added, filled with water up to 100 ml and incubated for 1,5 hours at 30°C. Absorbance of resulting blue colored liquids was measured at 765 nm. Quantitative analysis is performed based on the standard calibration curve of gallic acid in methanol. The concentrations of gallic acid in the solution from which the curve was prepared were 50, 100, 150, 250 and 500 mg/L (y=9,0135x-0,1935, R^2=0,9869). The result is expressed as mg of gallic acid equivalent per gram of dry weight of sample (mg GAE/100 g edible part of fruit).

Total flavonoid content (TFC)

Total flavonoids content (TFC) was determined by 24 h precipitation reaction with formaldehyde[13]. The remaining phenolic compounds, evaluated as non-flavonoid content (TNFC), were determined according to the previously mentioned procedure for TPC determination. TFC was calculated as subtraction of TPC and TNFC. In a 50 ml flask 5 ml of sample, 5 ml 1:4 HCl and 2,5 ml of formaldehyde solution was added, incubated for 24 hours at room temperature and filtered.

Determination of total anthocyanins

The content of anthocyanins was evaluated by the pH differential method spectrophotometrically[14]. Anthocyanins showed a maximum absorbance at 520 nm by pH 1.0 when coloured oxonium form was dominant, whereas colourless hemiacetal form was dominant by pH 4,5. The difference in absorbance was proportional to the anthocyanins content. Two solutions of fruit extract were prepared, one with potassium chloride buffer (0,2 M KCl) pH 1,0 and the other with sodium acetate buffer (1,0 M Na-Ac) pH 4,5 diluting each by the determined dilution factor of 1:20 (v/v). After 15 min incubation at room temperature the absorbance of each solution was measured at 514 nm and at 700 nm. The absorbance of the diluted samples is calculated as follows:

$$A = (A_{514\ nm} - A_{700\ nm})_{pH1.0} - (A_{514\ nm} - A_{700\ nm})_{pH\ 4.5}$$

$$\text{Antocyane (mg)} = \frac{A \times MW}{\varepsilon} = \frac{A \times 449{,}2}{29600} = \frac{A \times MW}{\varepsilon} = \frac{A \times 449{,}2}{29600}$$

$$\text{Total antocyane (mg/100 g fruit)} = \frac{\text{Antocijane (mg)} \times F \times V\ (\text{ml}) \times 100\ \text{g}}{\text{sample mass (g)}}$$

where is:

F=dilution factor (1:20)
V=volumen for solution for extraction (ml)

Determination of antioxidant capacity using DPPH (2,2-diphenyl-1-picrylhydrazyl) method
In the DPPH method[15], reaction solution was prepared by mixing 50 µl of diluted extract with 0,5 ml of 0,5 mM solution of DPPH and filled by methanol to 2 ml. The mixture was incubated for 30 min in the dark at room temperature. The absorbance was measured against the prepared blank (50 ml diluted fruit juice, 2950 ml methanol) at 517 nm. For DPPH blank solution preparation 1 ml of 0,5 mM DPPH solution was dissolved with 4 ml of methanol. Percent inhibition of DPPH radical is calculated according to the equation:

$$\text{Radical scavenging effect}\ (\%) = \left[1 - \frac{\text{absorbance of sample}}{\text{absorbance of blank}}\right] \times 100$$

Results are expressed as the IC 50 value (mg/ml) or the concentration of sample that causes 50% neutralization of DPPH radicals.

HPLC method

HPLC analysis of phenolic components in extracts was performed on an Agilent Series 1100

HPLC-system equipped with an Agilent 1200 Series DAD detector. The liquid samples filtered with 0,45 mm filter was introduced into the HPLC system using the Agilent 1100 Series auto sampler. System management and processing chromatograms was performed using Agilent LC Chem-Station software for data analysis. For HPLC analysis Zorbax SB C18 column was used, mobile phase ethanol/water 20/80 % v/v, flow rate 0,4 ml/min, detection: 254 nm (520 nm), injected volume 20 ml, room temperature[16]. As reference standards solution cyanidin-3-rutinoside chloride and cyanidin-3-glycoside chloride were used.

RESULTS AND DISCUSSION

Quantitative measurements of total phenols are based on the standard calibration curve of different concentrations of gallic acid. The results of determining TPS in the analyzed samples of fresh sour cherries and processed cherries are shown in the Table 1.

Table 1. Content of total phenols, flavonoids and anthocyanins in fresh cherry, juice, concentrate, extra jam and lyophilised cherry

Sample	Total phenols (mg GAE/100 g)	Total flavonoids (mg/100 g)	Total anthocyanins (mg/100 g)
Cherry	179,5 ± 9,7	37,5 ± 2,1	82,2 ± 7,3
Juice	199,7 ± 5,4	35,7 ± 3,8	22,4 ± 2,9
Concentrate	44,9 ± 7,6	7,5 ± 3,3	17,7 ± 5,7
Extra jam	116,7 ± 3,1	33,8 ± 5,4	30,1 ± 4,8
Lyophilisate	130,3 ± 8,6	38,7 ± 6,3	47,5 ± 7,8

The results show that fresh cherry has a relatively high content of phenol (179,5 mg GAE/100 g edible part of the fruit cherries). The result is consistent with the literature data[17, 18]. The total phenol content in the products obtained from processing of sour cherries shows that cherry juice has maintained a high phenolic content (199,7 mg GAE/100 g), while the amount of TPC is somewhat preserved in lyophilized cherries (130,29 mg GAE/100 g). The production process of extra jam reduces TPC and this value is 116,67 mg GAE/100 g. During the process of concentration of cherry juice there is a significant loss of TPC (44,9 mg GAE/100 g). It can be concluded that the vacuum processing of cherries greatly influences the value of TPC. Total flavonoids are calculated from the difference between total phenol and non-flavonoid content. It can be seen from Table 1 that the highest content of flavonoids have fresh cherries, followed by freeze-dried cherry, cherry juice and extra jam (33,75 mg GAE/100 g). The results show that lyophilisation process preserves the content of flavonoids. The flavonoid content of cherry juice is much higher compared to the concentrated juice, which means that the process of vacuum concentrating causes a loss of flavonoids. Process of jam production where high temperature is applied do not greatly influence the content of flavonoids.

The content of anthocyanins has long been used as to assess adulteration by the addition of juices from different fruit sources[19]. The profile of anthocyanins is characteristic for different types of fruits and all these variations affect the way of determination. Table 1 shows the results of total anthocyanins per 100 g of sample. Anthocyanins are quite reactive and unstable compounds. The highest content of anthocyanins is measured in fresh cherries (82,2 mg/100 g edible part). The results are similar with the results obtained by other authors[20]. Lyophilisation of cherries most preserves the anthocyanins content (47,50 mg/100 g). Because the anthocyanins loss may occur in the presence of oxygen and at elevated

temperatures, cherry processing causes the reduction of the content of anthocyanins. The lowest content of anthocyanins is registered in the concentrated juice and amounts to 17,70 mg/100 g sample.

Antioxidant capacity depends on the composition of the extract and methods that are used for their determination. DPPH is a stable radical and is often used to assess the antioxidant capacity of different natural products. The results in Figure 1 are expressed as the IC 50 value (mg/ml) or the concentration of sample that causes 50% neutralization of DPPH radicals.

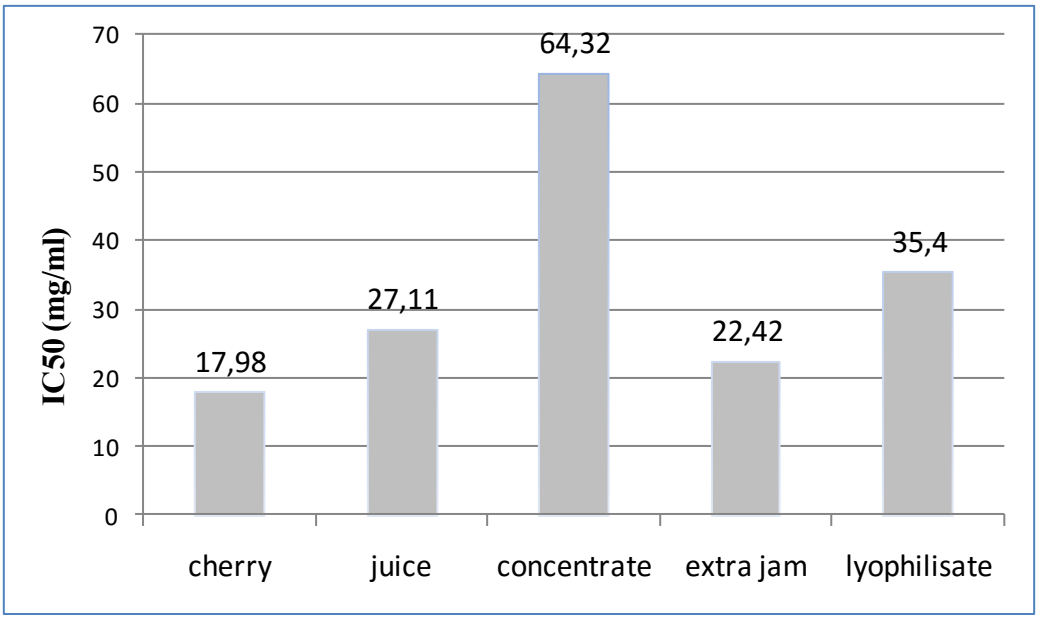

Figure 1. Antioxidant capacity of cherries and processed cherries determined by DPPH method

The results in Figure 1 show that all samples have similar values of antioxidant capacity or mass of the sample needed to achieve 50% inhibition of DPPH radicals. The cherry concentrate shows the weakest antioxidant capacity (64,32 mg/ml). It should be considered that fresh cherry has a significant content of vitamin C which is one of the most powerful antioxidants. During the technological process, especially during the preparation of concentrate by evaporation, part of vitamin C is lost, which lowers antioxidant capacity of the concentrate.

Reversed phase RP-HPLC is the most used method for detection and identification of anthocyanins. Anthocyanins could be separated according to their polarity and detected on the chromatogram at different retention times. Most of methods for the analysis of anthocyanins are qualitative or semi-quantitative and provide information about identification of anthocyanins in their source. The big challenge is the quantification of anthocyanins by HPLC due to the absence of most commercial standards. The composition of anthocyanins in all samples was analyzed by HPLC (Figure 2). Cyanidin-3-rutinosid and cyanidin-3-glycoside are used as reference standards. Retention time (RT) of cyanidin-3-glycoside is 7,55 min and retention time of cyanidin-3-rutinosid is 7,86 min.

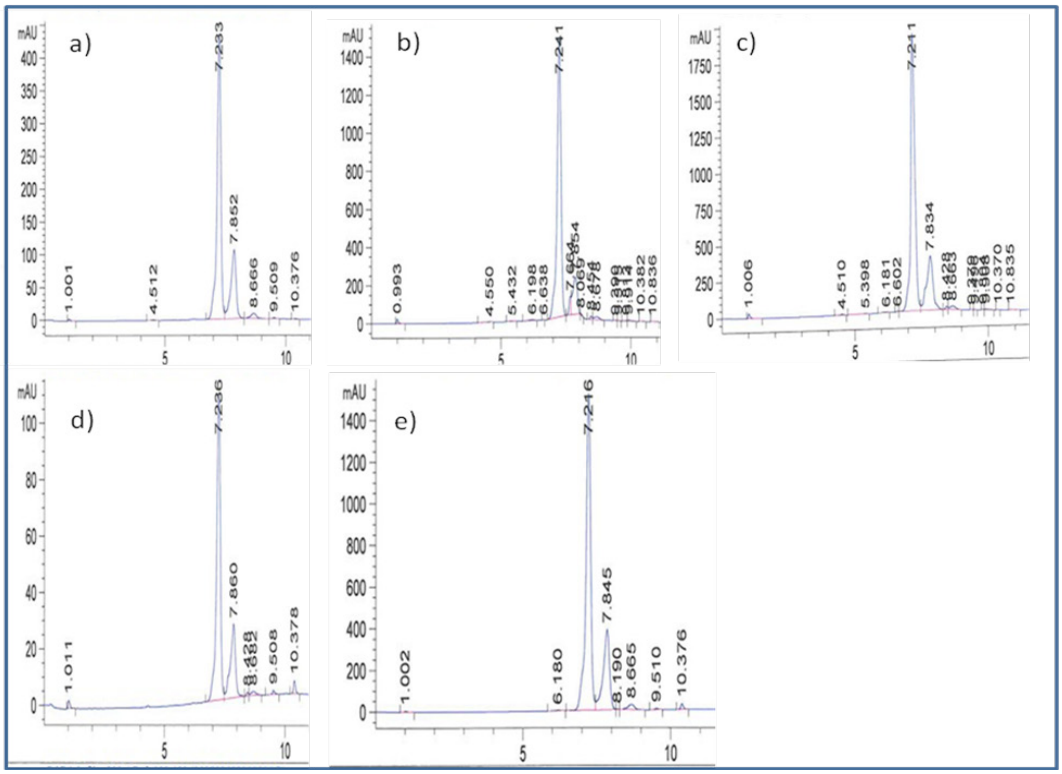

Figure 2. HPLC diagrams of anthocyanins profile of fresh cherry (a), juice (b), concentrate (c), extra jam (e) and lyophilised cherry (d)

On HPLC diagrams of all samples (Figure 2) there is a peak detected at RT 7,86, which corresponds to cyanidin-3-rutinosid. None of the sample diagrams show peak at RT 7,55, which corresponds to cyanidin-3-glycoside. However, the intensity of this peak is different, where juice, concentrate and lyophilisate show the strongest intensity. Since the peak intensity corresponds to the amount of anthocyanins in the sample, it can be concluded that the technological process of production of jam causes the loss of anthocyanins. In all diagrams there is the high intensity peak at RT 7,24 min detected, which cannot be identified. Also, several other peaks of lesser intensity are registered that, due to the lack of a reference standard, are not possible to identify.

CONCLUSION

Since the trend of manufacturing of value added products in the world is increasing, there is need to affirm application of sophisticated technology such as freeze-drying and vacuum concentration in order to increase the competitiveness of food products at the local level in Bosnia and Herzegovina and in such way to increase the export potential of these products. The aim of this study was to demonstrate the effect of different ways of cherries processing technologies on the content of bioactive compounds, especially of total phenols, flavonoids and anthocyanins. Also, the impact of processing on the antioxidant capacity of cherries and processed cherries was compared. The analysed products were obtained by lyophilization (lyophilisate) and vacuum concentration (concentrate) and compared to the content of bioactive components and antioxidant capacity of these products with the product obtained by conventional processes (extra jam).

The results showed that the processes of cherries affect the content of bioactive ingredients. This study showed that the greatest influence on the

content of bioactive ingredients has process where vacuum concentration is applied. Conventional technologies used for production have a negative impact on the bioactive components. The use of more sophisticated technologies such as lyophilization would contribute to the retention of bioactive components and in that way increase product competitiveness and at the same time meet constant market growth for functional foods that is rich in antioxidants.

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EFFECT OF ADDITION OF TREHALOSE, MALTOSE AND TWO MODIFIED STARCHES ON COLOUR AND TEXTURAL ATTRIBUTES OF CHICKEN SURIMI GELS DURING FROZEN STORAGE

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

Texture profile analysis (TPA) and instrumental colour parameters of chicken surimi gels after frozen storage were investigated. Chicken surimi gels were prepared from mechanically deboned chicken meat, mixed with trehalose (*w* = 8%), maltose (*w* = 8%), tapioca modified starch (MTS) (*w* = 8%) and barley modified starch (MBS) (*w* = 8%), quickly frozen and stored for 90 days s on -30°C. Instrumental colour parameters (Lightness (L*), redness (a*), yellowness (b*) and whiteness (L* − 3b*) of chicken surimi gels were significantly (P < 0,05) affected by addition of trehalose, maltose and modified starches. Highest values of lightness (L*) and whiteness showed sample mixed with MBS. Textural profile analysis (TPA) parameters, hardness and chewiness increased significantly (P < 0,05) by addition of trehalose, maltose, MBS and MTS. Cohesiveness and springiness of chicken surimi gels were also significantly (P < 0,05) affected by addition of trehalose, maltose MBS and MTS. Increase in colour and textural attributes of chicken surimi gels after frozen storage indicates possible interactions between chicken myofibrillar proteins and trehalose, maltose and modified starches.

Key words: Chicken surimi, Texture, Colour, Modified starch, Trehalose, Maltose, Frozen storage.

INTRODUCTION

Chicken myofibrillar protein concentrate, produced with modified technology used for fish surimi[1,2], is characterized by very good technological properties, such as a high water holding capacity and a high ability to form strong gels after being heated[3]. Freezing has become the most frequently used preservation method for meat and meat products. To protect myofibrillar proteins from denaturation during frozen storage and maintain its possible high processability, some cryoprotectants (i. e. disaccharides, polysaccharides, polyalcohol's, organic acids and polyphosphates) are generally added[4,5].

Trehalose (D-glucopyranosyl-α (1→1)-D-glucopyranoside) is a non-reducing disaccharide with low caloric value and low sweetness, which is only 45% of that of sucrose[6]. Because of its ability to form strong hydrogen bonds with the polar group of the biomolecules and higher glass transition temperature, trehalose has superior preservation properties as compared to other sugars[7]. Trehalose has been found to have a protective effect against thermal inactivation of enzymes and freeze-draying of microorganisms[8]. Osako et al.[9] investigated gel forming ability, unfrozen water content, Ca^{2+} ATPase activity of horse mackerel surimi, during freezing and frozen storage, upon addition of trehalose, sucrose, glucose and sorbitol and concluded that trehalose had similar cryoprotective effect as sucrose and sorbitol.

Colour and texture are the major factors responsible for the final acceptance of surimi-based products by consumers[10]. To better suit the textural preferences of consumers, ingredients must be added to surimi that modify the textural and water mobility properties of the surimi[11]. In composite food such as surimi the additives can modify the texture. Protein additives, such as egg white, are used to increase the gel strength and to give a whiter and glossier appearance to the gel[12]. The final surimi-based product can assume almost any desired texture through its gel forming capacity.

Starch is usually added to surimi to improve gelling properties and final textural properties [13].

Gelation of starch is thought to arise from the formation of a three dimensional network which binds the swollen granules. Factors involved in the gelatinization of starch are mainly type, size and previous history of starch granules[14], concentration, temperature, cooking time, agitation, types, and amounts of added ingredients. Starch gelatinization is accompanied by melting of crystallites, loss of birefringence, granule swelling, and increase in viscosity[11].
The objective of this study was to determine influence of trehalose, maltose, MTS and MBS on instrumental colour and texture parameters of chicken surimi gels after frozen storage.

MATERIAL AND METHODS

Sample preparation

Chicken surimi samples were prepared in the laboratory from mechanically deboned chicken meat using the modified procedure of Yang and Froning[15] where washing and leaching was performed with distilled water, instead of with tap water. The samples were mixed with trehalose, maltose, modified tapioca starch (MTS) and barley starch (MBS) in mass fractions 8%. Mass fractions were determined as percent of total mass. MBS modification procedure: 0,145 g glutaric acid was suspended in 4,35 g acetic anhydride. 100 g of starch (d.m.) was suspended in 145 ml distilled water by agitating at magnetic agitator (300 rpm), during agitating the solution of glutaric acid and acetic anhydride was added. pH of suspension was kept at 9 with 1M NaOH. After addition of modified agents the stirring was continued for another 30 min. The reaction of starch with glutaric acid and acetic anhydride was terminated by reduction of pH to 5 with addition of 1M HCl. Suspension was than centrifuged (300 rpm/5min) to separate starch. Starch pellet was resuspended in water and centrifuged again. This step was repeated until supernatant became colourless. Starch suspension was then neutralized, centrifuged once more and modified starch was finally air dried. MTS was prepared according to the method by Wurzburg[16]. The samples were packed in plastic test tubes with an inside diameter of 10 mm, frozen, and stored at -30 °C. Texture profile analysis and colour parameters were evaluated after 90 days. Water activity (a_w) was determined using a Rotronic Hygrolab 3 (Rotronic AG, Bassersdorf, Switzerland) at a room temperature (20 ± 2ºC). The Food Scan Meat Analyser was used to determine moisture, total protein share, total fat share and collagen content according to the AOAC 2007. 04[17].

Texture profile analysis (TPA)

After defrosting, test tubes with their content were heated for 25 min in a water bath at 80°C. The test tubes with produced gels were cooled in ice water until the temperature of approx. 20°C was obtained inside the sample. After that, they were stored at 4 - 6°C until the next day. Texture profile analysis (TPA) tests were performed using a TA.XT2i SMS Stable Micro Systems Texture Analyzer (Stable Microsystems Ltd., Surrey, England) equipped with a cylindrical probe P/75. This involved cutting samples into 1,5 cm thick slices and compressed twice to 60% of their thickness. Force-time curves were recorded at across-head speed of 5 mm/s and the recording speed was also 5 mm/s. The following parameters were quantified [18]: hardness (g), maximum force required to compress the sample, springiness (ratio), the ability of the sample to recover its original form after the deforming force was removed, cohesiveness, extent to which the sample could be deformed prior to rupture (ratio) and chewiness (g), which is calculated hardness · cohesiveness · springiness, was measured.

Determination of colour

Colour measurements (L*, a*, and b* values) were preformed using a Hunter-Lab Mini ScanXE (A60-1010-615 Model Colorimeter, Hunter-Lab, Reston, VA, USA). The instrument was standardized each time with a white and black ceramic plate (L*0 = 93,01, a*0 = -1,11, and b*0 = 1,30). The Hunter L*, a*, and b* values correspond to lightness, greenness (-a*) or

redness (+a*), and blueness (-b*) or yellowness (+b*), respectively. The whiteness (W) was calculated using the formula: L* - 3 b*. The colour measurements were performed on chicken surimi at a room temperature (20 ± 2 ºC).

Statistical analysis

Three determinations for basic chemical composition, seven for TPA and colour parameters were measured from each sample. Experimental data were analysed by the analysis of variance (ANOVA) and Fisher's least significant difference (LSD), with significance defined at P< 0,05. Statistical analysis was carried out using the Statistica ver. 12.7.StatSoft Inc. Tulsa, 2015. OK. USA.

RESULTS AND DISCUSSION

The mean basic chemical composition, pH and a_w values of chicken surimi samples before mixing with sugars or modified starches are presented in Table 1. The mass fraction of water, protein and fat in chicken surimi were similar to the results reported by Stangierski and Kijowski[19] for myofibril concentrate prepared from mechanically recovered poultry.

The TPA parameters of chicken surimi mixed trehalose, maltose, MTS, and MBS after 90 days of frozen storage are shown in Table 2. Four parameters were obtained: hardness, springiness, cohesiveness and chewiness. The chicken surimi samples frozen for 90 days had higher values for TPA parameters then fish surimi reported by Tablio-Munizaga and Barbosa-Canovas[20]. This can be attributed to the different nature of samples (chicken and fish meat).

Table 1. Basic chemical composition, a_w and pH of chicken surimi samples

Water *w* (%)	Proteins *w* (%)	Fat *w* (%)	Collagen *w* (%)	pH	a_w
84,75± 0,28	13,06± 0,58	0,73± 0,07	0,79± 0,01	6,95± 0,04	0,98± 0,01

Values are means ± Standard deviation of triplicate.

The sample of chicken surimi without addition of additives showed lowest values of hardness and chewiness. The hardness of chicken surimi gels increased significantly (P< 0,05) with the addition of 8% trehalose, 8% maltose, 8% MBS and8% MTS. The chicken surimi hardness and chewiness were more influenced by addition of sugars then by the addition of modified starches.

Table 2. Texture profile analysis parametrs of chicken surimi mixed with 8% trehalose, 8% maltose, 8% MBS and 8% MTS after 90 days of frozen storage

	Hardness	Springiness	Cohesiveness	Chewiness
No additive	105,04d ± 12,72	0,89ab ± 0,02	0,74b ± 0,04	69,86 ± 12,92
Trehalose	274,61a ± 27,32	0,87ab ± 0,01	0,77a ± 0,01	184,10a ± 14,48
Maltose	221,98b ± 28,17	0,85b ± 0,01	0,77a ± 0,03	146,41b ± 8,37
MTS	201,13b ± 20,52	0,93a ± 0,05	0,72b ± 0,01	135,81b ± 11,26
MBS	135,60c ± 9,32	0,88ab ± 0,01	0,79a ± 0,01	94,85c ± 14,51

Values are means ± SD seven measurements. Values in the same row with different superscripts (a-c) are significantly different (P<0,05).

The springiness of chicken surimi samples were in the range from 0,85 to 0,93. The highest values of springiness showed the sample mixed with 8% MTS. Chicken surimi samples cohesiveness was significantly (P<0,05) affected with addition of sugars and modified starches (Table 3). The

main differences in TPA among different additives added were obtained in hardness and chewiness. The increase of texture profile analysis parameters with the addition of sugars and modified starchesare was in agreement with the result reported by Alakhrash et al.[21], for fish surimi with addition of oat bran.

Instrumental colour parameters of chicken surimi with addition of sugars and modified starches are presented in Table 3. Generally, the demand is higher for surimi gels with high lightness (L*), low yellowness (b*) and high whiteness (W). The colour parameters of chicken surimi were different then the colour parameters of fish surimi[20, 22]. This can be related to the nature of sample (higher myoglobin content in chicken meat). Similar, higher values for L*, a*, b* for pork and chicken surimi in comparison with Alaska Pollock surimi were reported by Jin et al.[23]. The addition of trehalose, maltose, MBS and MTS significantly increased (P< 0,05) lightness and whiteness of chicken surimi samples. This is in agreement with the studies that investigated the addition of potato starch and egg white and oat bran to Alaska Pollock surimi[20, 21]. The addition oftrehalose, maltose, MBS and MTS to chicken surimi, resulted in decreased of a* values, indicating a slightly greater green hue in these treatments. Yellowness (+b*) decreased with the addition of oats 8% trehalose, maltose, MBS and MTS. This decrease was higher in samples of chicken surimi mixed with sugars then modified starches.

Since the most important quality parameter in surimi is whiteness, and in order to better predict the behaviour of additives, whiteness was calculated according to the formula W = L* - 3b*. The whiteness of chicken surimi samples varied from 20,62 to 33,45. The similar increase in lightness and whiteness for the heat induced fish surimi gels mixed with potato starch and egg were reported by Tabilo-Munizaga and Barbosa-Canovas[20]. The addition of sugars and modified starches significantly increased whiteness (P< 0,05) of chicken surimi samples.

CONCLUSIONS

The results of this study showed statistically significant (P < 0,05) increase of lightness (L*) and whiteness (W), decrease of greenness (+a) and yellowness (+b) of chicken surimi samples with addition of trehalose, maltose and two modified starches. Also, the increase of TPA parameters of chicken surimi samples after frozen storage with the addition of trehalose, maltose and two modified starches were observed. This can indicate possible interaction of trehalose, maltose, MBS AND MTS with the chicken myofibrillar proteins and its stabilisation.

Table 3. Instrumental colour parameters of chicken surimi mixed with 8% trehalose, 8% maltose, 8% MBS and 8% MTS after 90 days of frozen storage.

No additive	L*	a*	b*	Whitness L* – 3b*
No additive	75,70e ± 0,28	2,38a ± 0,12	18,36a ± 0,39	20,62c ± 1,03
Trehalose 8%	79,58c ± 0,20	1,91ab ± 0,15	15,66ab ± 0,31	30,62b ± 1,11
Maltose 8%	77,22d ± 0,89	1,63b ± 0,26	15,53b ± 0,36	31,51ab ± 1,90
MTS 8%	80,70b ± 0,23	2,21a ± 0,16	16,41b ± 0,59	32,59ab ± 1,57
MBS 8%	82,49a ± 0,44	1,72b ± 0,07	16,35b ± 0,29	33,45a ± 0.50

Values are means ±SD of seven measurements. Values in the same row with different letters (a-b) are significantly different (P< 0,05)

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INFLUENCE OF ANTHROPOGENIZATION ON THE SOIL PROPERTIES DEVELOPED ON SILICATE SUBSTRATES IN THE WESTERN PART OF BOSNIA AND HERZEGOVINA

PROFESSIONAL PAPER

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ABSTRACT

This paper analyzes the natural and anthropogenized soils on silicate substrates in the western part of Bosnia and Herzegovina. The aim of the research is to evaluate the impact of anthropogenization on these soils, using a method of comparing physical and chemical properties. The research was conducted in four locations in Bosanska Krajina by examining the following soil subgroups of Dystric Cambisol: Acidic brown soil on micaschist at site Šabići; Acidic brown soil on schist (Dystric Cambisol) at site Donja Lučka; Acidic brown soil on shales, site Jusufovići; Acidic brown soil on sandstone, site Baštra. In each of these sites, two profiles were opened; one on natural soil and others on anthropogenic - arable land, while the samples were taken in the profile on the horizon. We analyzed the following indicators of soil quality: soil organic matter content, pH, content of available forms of P_2O_5 and K_2O, analysis of soil adsorption complex by Kappen method. The aim of the research is to determine differences in some soil properties between anthropogenic and corresponding soils under natural forest or meadow. By comparing the sample results of the analysis from the horizons of natural and cultivated soils, it was concluded that the proper application of agro-technical measures and agro-ameliorative measurements generally did not cause negative effects on the properties of these soils, and often showed a positive impact.

Keywords: chemical properties, use, anthropogenization, soil properties.

INTRODUCTION

In the region of Bosanska Krajina so far mainly two types of soil have developed on carbonate and silicate substrates, which are distributed throughout the region as a mosaic.
On the basis of carbonate substrates (limestone and dolomite) various types of soils have developed; varying in type, origin, depth, humus content and general agro-ecological characteristics.
Silicate substrates on the other hand, are throughout the region of Bosanska Krajina mainly represented by a second type of soils: aleurolites, sandstone, clay and shale. These substrates make the basis for soil more uniform in physical and chemical properties, which are generally characterized by greater depth, acidity and generally favorable water-air regime. The influence of mankind on the land, developed on the basis of silicate substrates, has left significant consequences. Namely, by continued deforestation and plowing of natural meadows in order to create arable land, mankind has changed the vegetation layer and its function as a natural ecosystem.

MATERIAL AND METHODS

This paper presents the reaserch undertaken on anthropogenized and natural soil formed on silicate substrates in the area of Bosanska Krajina. Throughout the sampling process, a soil map of B&H scaled 1:50,000 was used, and for each type of soil, two profiles were sampled. One was collected from the arable-anthropogenic soil and the other from the natural soil, which development was not affected by the influence of mankind.

The profiles were taken up to from a depth of 100 cm, in individual samples all but three replications, undisturbed samples in Kopezky rings and disturbed samples in plastic bags, and this paper illustrates the average data results from the laboratory studies, which were carried out using the standard methodology (Enger-Riehm-Domingo method). The samples were used for the determination of organic matter content, soil pH, available P_2O_5 and K_2O, and to analyze the soil adsorption complex (SAC).

RESULTS AND DISCUSSION

Acidic brown soil found on mica schists, site Šabići

The acidic brown soil, found on mica schists, was sampled on the location of Šabići in Cazin (located to the left of the regional road Cazin-Velika Kladusa) and consequently tested. A large number of the regional population is occupies it self with agricultural production, and despite the hilly terrain, a large number of plots are intensively farmed. A profile of the natural soil was taken from the ground used for pasture, never previously having been under the influence of anthropogenization. The profile was established on a plot of 0,8 ha, which is situated below the forest belonging to the Quercus cerris family, (Turkey oak or Austrian oak). Another profile was taken of the anthropogenic soil in the garden of 0,2 ha in size, located on a gentle slope under the Quercus cerris trees, about 100 m distance away from the family home. This plot of land has been used for gardening approximately for 7 years. Every fall, the basic agricultural processing (plowing) occurs, and prior to it, about 2 tons of manure is distributed equally per parcel. The results of the analysis are shown in Table 1.

Table 1. Chemical properties of the soil, site Šabići

Use	Horizon	Depth in cm	Humus %	pH		P_2O_5	K_2O
				H_2O	1MKCl	mg/100g soil	
Natural soil	A	0 – 17	1,8	5,3	4,3	1,4	11,5
	Bv	17 – 60	0,4	5,6	4,2	0,8	5,7
	BC	60 -100	0,1	5,3	4,7	1,1	9,7
Arable land	Ap	0 -33	3,3	6,3	5,2	1,6	25,6
	Bv	33 – 75	0,5	6,0	4,4	1,7	7,6
	BC	75 - 100	0,2	6,0	4,5	1,0	12,6

The Ap-horizon in the anthropogenic soil spreads throughout the depth of 33 cm, a result of tillage at a certain depth, where after a while Ap-horizon had formed. The A-horizon in the natural soil however, continues at a much shallower depth of 17 cm. The humus content measured within the anthropogenic soil was highest in the Ap-horizon (3,3%), whereas it was measured only 1,8% in the A-horizon within the natural soil. The increasing amount of humus in the Ap-horizon is attributed to the anthropogenic activities, or to adding manure to the soil, a trait common to every anthropogenic horizon found in plots of land used for farming or gardening purposes. The value in the Ap-horizon is moderately acidic, and in natural soil under grazing land, very sour. The pH value in H_2O in the Ap-horizon belonging to the anthropogenic soil lies in the range of 6,2 to 6,4 while the pH value in H_2O in the A-horizon of the natural soil is somewhat lower, falling into the range of between 5,3 and 5,6. The reason for this difference in pH values between the same soil types is simply land use, and introduction of organic and mineral fertilizers to the land used for gardening, for the purpose of adding nutrition to the growing vegetables. There was no difference in the content of available phosphorus, and the values were in the range of 1,7 - 10 mg P_20_5/100 g soil. The potassium content was the highest in the Ap-horizon of the anthropogenic soil, at 25,6 mg potassium per 100 g soil, twice as higher than at the same depth of natural soil. These differences in the content of potassium can be viewed as a result of fertilization with mineral and organic fertilizers. The results of the analysis are shown in Table 2.

Table 2. Analysis of the soil absorption complex according to Kappen

Use	Depth in cm	Hydrolytic acidity (H)	Content of exchangeable bases (S)	Total absorption capacity (T)	Degree of base saturation related to Total absorption capacity (V)
		$cmol/kg^{-1}$	$cmol/kg^{-1}$	$cmol/kg^{-1}$	%
Natural soil	0 - 10	8,1	16,4	24,5	66,9
	10 - 20	7,1	14,7	21,8	67,4
Arable land	0 - 10	2,9	21,5	24,4	88,0
	10 - 20	3,2	17,5	20,7	84,3

When talking about the properties of soil adsorption complex by Kappen method, the hydrolytic acidity (H) is higher in the natural soil than in the anthropogenic soil. Furthermore, the degree of base saturation (V) in the anthropogenic soil throughout the depth of 0 to 20 cm is considerably higher, than in the natural soil. Since the pH reaction values, observed in the anthropogenic soil, are more favorable, than the ones in non-arable soil, we can deduce that these parameters indicate a positive anthropogenic influence on the land used for gardening. As by definition, brown soil, found in the region, has a pH value of 5,5 below water and degree of saturation below 50%, and due to its specific features, we opted for a term used in the pedological map of BiH, when classifying this type of soil.

Acidic brown soil found on schists, site Donja Lučka

The village of Donja Lučka is located on the border of the municipality of Cazin and Velika Kladusa, to the left of regional road Cazin to Velika Kladusa. The geographical profile varies between being hilly to mountainous, and large areas of the region are used for agricultural purposes, as arable land. A profile of the natural soil was sampled at the top of the slope, which falls at about 15%. The entire plot is covered by a layer of moss of 2 to 5 cm thickness, and on the east side of it, an oak and beech forest is located, where the water source is much poorer. The profile of the anthropogenic soil was collected on the plot of 1 ha, which is located at the bottom of a slope, just below the oak and beech tree forest, on the left side of main road Cazin to Velika Kladusa. The terrain has a slight decrease of 3%, and is used for farming purposes. During wheat sowing months, basic fertilization (10 tons of cattle manure) and basic farming tools, are used. The owner stated that the crop's growth progress was amplified by using four bags (200 kg) of KAN 27% fertilizer. No other chemical were used, or introduced in any other form, to this plot of land. The results of the analysis are shown in Table 3.

Table 3. Chemical properties of the soil, site Donja Lučka

Use	Horizon	Depth in cm	Content measured in organic matter %	pH		P_2O_5	K_2O
				H_2O	1MKCl	mg/100g soil	
Natural soil	A	0 – 29	2,9	5,2	4,2	2,3	15,1
	Bv	29 - 64	0,4	4,8	3,8	0,3	7,4
	BC	64 - 100	0,2	4,6	3,6	1,8	10,0
Arable land	Ap	0 – 20	2,9	6,4	5,5	3,0	18,7
	Bv	20 - 54	0,8	6,1	5,2	2,6	8,6
	BC	54 - 100	0,4	5,8	4,6	0,7	8,5

The pH value in 1 M KCl measured in the anthropogenic soil ranges between 5,5 within the Ap-horizon to 4,6 in the BC-horizon, while the pH value in 1 M KCl measured in the natural soil ranges between 4,2 in the A-horizon to 3,6 in the BC-horizon. The cause for the noticeably higher pH values in the anthropogenic soil may be found in the nature of the paternal substrate which can be mosaic in small intervals, as well as in the land use, meaning, the use of mineral fertilizer KAN 27% and manure. Observing the values of humus content measured in the anthropogenic and natural soil, one must conclude that these values are identical, which is still a good indicator that no reduction of organic matter, caused by tillage (an occurrence symptomatic of adding natural fertilizer/manure to the soil), has occurred. The content of physiologically active phosphorus in the anthropogenic and the natural soil are almost identical, and the differences in the potassium content are small as well. The results of the analysis are shown in Table 4.

Table 4. Chemical properties of the soil absorption complex according to Kappen, site Donja Lučka

Use	Depth in cm	Hydrolytic acidity (H)	Content of exchangeable bases (S)	Total absorption capacity (T)	Degree of base saturation related to Total absorption capacity (V)
		cmol/kg^{-1}	cmol/kg^{-1}	cmol/kg^{-1}	%
Natural soil	0 – 10	10,0	14,7	24,8	59,3
	10 – 20	9,1	17,5	26,6	65,8
Anthropogenic soil	0 – 10	3,5	3,8	7,3	51,6
	10 – 20	5,5	2,7	8,3	33,5

The values of H, S and T are twice as high in the natural soil, than in the anthropogenic soil. The degree of base saturation in the anthropogenic soil measured 51,6% at the depth of 0 to 10 cm, and 33,5% at the depth of 10 to 20 cm. When it comes to natural soil, values vary, so the degree of base saturation measured 59,3% at the depth of 0 to 10 cm and 65,8% at the depth of 10 to 20 cm.

Acidic brown soil found on clay schist, site Jusufović

The village of Jusufović is located in the municipality of Bužim, just left from the main road Cazin to Bužim. The geographical profile varies from hilly to mountainous, and the land is covered by forests or natural grazing land. The soil is characterized by low production capacity and the erosion in this area is very emphasized due to the high inclination of the field. A profile of the natural soil was taken from a clearing located 50 m distance from the oak tree forest, which lies to the west at the very top of the hill. The forest is sparsely populated by relatively thin trees, indicating a younger forest, and because their roots consist of densely knotted thin and thick vessels, making the soil hard, it made the sampling of the soil profile somewhat problematic. A second soil profile was sampled in the garden, located beneath the oak tree forest. This plot of land is strictly used for agricultural purposes, and one of the mandatory tillage measures is certainly basic fertilization using manure (about 2 t per ha), and plowing conducted with a tractor plow at a depth of about 25 cm. The results of the analysis are shown in Table 5.

Table 5. Chemical properties of the soil, site Jusufovići

Use	Horizon	Depth in cm	Content measured in organic matter %	pH		P_2O_5	K_2O
				H_2O	1MKCl	mg/100g soil	
Natural soil	A	0 – 12	2,6	4,3	3,3	1,0	36,1
	ABv	12 – 39	1,5	4,6	3,4	0,7	20,5
	Bv	39 – 80	0,7	5,1	3,4	0,2	19,7
Arable soil	Ap	0 – 23	2,0	6,2	5,5	5,3	129,0
	ABv	23 – 50	1,4	6,0	5,2	1,0	56,8
	Bv	50 - 100	0,3	5,7	4,3	0,4	9,6

Looking at the depth of the individual horizon, primarily the surface horizon, one can notice the difference in their depth. The plots of land used for gardening indicate a depth of 23 cm in the Ap-horizon, the same depth necessary for tillage process, while the soil beneath a forest indicates in much shallower depth of the A-horizon, with measured depth of 12 cm. Resulović et al. 2008, states that the acidic brown soil found in the forest makes the basis for a layer of litter ranging 2 to 4 cm in thickness, while the A-horizon is quite shallow, about 10 cm in depth, (in very few cases up to 30 cm). The pH values in 1M KCl are strongly acidic (pH 3,3-3,4) throughout the entire depth of the natural soil, while the pH values in the soil, found on the plot used for gardening, indicate lesser, though still strong, acidity; being moderately acidic in the Ap and the ABv- horizon, and highly acidic in the BC-horizon.

It can be deduced therefore that the favorable pH values observed in the soil, found on the plot of land used for gardening purposes, is a positive reaction to mankind influence and activity in comparison to natural soil found beneath the forest area. The humus content within the surface horizon was observed to be higher on the forest soil, measuring 2,6%, in comparison to the 2,0% measured on the plot of land used for gardening purposes. However, it is important to note that the Ap-horizon spreads 23 cm in depth throughout the plot used for gardening purposes, whereas the A-horizon spreads 12 cm in depth throughout the forest soil which ultimately means a more uniform amount of carbon.

The content of available phosphorus varied in values, thus the maximum values (5,3 mg per 100 g soil) were measured in the anthropogenic soil throughout the Ap-horizon, while the values were at its lowest (1,0 mg per 100 g soil) within the A-horizon belonging to the natural soil. The anthropogenic activities have contributed to increased potassium content in the soil on the plot of land used for gardening purposes, indicating thus values of 129,0 mg per 100 g soil in the Ap-horizon belonging to the soil on the plot of land used for gardening purposes, whereas the values (36,1 mg per 100 g soil) measured significantly lower on the surface horizon of the forest soil. The results of the analysis are shown in Table 6.

Table 6. Chemical properties of the absorption complex according to Kappen, site Jusufovići

Use	Depth in cm	Hydrolytic acidity (H)	Content of exchangeable bases (S)	Total absorption capacity (T)	Degree of base saturation related to Total absorption capacity (V)
		$cmol/kg^{-1}$	$cmol/kg^{-1}$	$cmol/kg^{-1}$	%
Natural soil	0 - 10	18,5	8,3	26,8	31,0
	10 - 20	15,2	8,3	23,6	35,3
Anthro-pogenic soil	0 - 10	1,3	38,2	39,5	77,3
	10 - 20	0,9	17,1	18,0	76,5

The degree of base saturation in the anthropogenic soil at a depth of 0 to 10 cm is 77,3%, while the values at the second depth of 10 to 20 measure 76,5%. In the soil found on the forest soil these values are not as high, so at a depth of 0 to 10 cm, the degree of base saturation is observed to be at 31,0%, while at the depth of 10 to 20 cm it is 35,3%. Thus, by comparing the soil found on the plot of land used for gardening purposes and the soil found on the forest soil, it can be concluded that the anthropogenic measures have had a positive impact on increasing the values S, T and V.

Acidic brown soil found on sandstone, site Baštra

The acidic brown soil, found on sandstone, the sample was taken in the village of Baštra (belonging to the municipality of Bosanska Krupa, just to the right of the regional road of Bosanska Krupa - Bužim) and then tested. The geographical profile of the area varies from hilly to mountainous. The soil formed on sandstones is characterized by being well water absorptive, well aerated and poorly retaining water and nutrients. The soil on this location tends to heat up due to the low water capacity in the spring. For the geographic profile, cultivated fields are characteristic, while meadows are less represented. The plot on which the natural soil profile is open is distant from the village about 500 m, covered with woody shrubs such as birch, bracken, blackberry, which makes the plot unsuitable for sheep grazing, which otherwise occurs throughout the year. When sampling the profile, it was taken into a count that the surface layer of the soil is covered with a layer of moss, 3 to 5 cm thick. A profile of the anthropogenic soil was taken from a pit at the bottom of the slope, just 50 m below the chestnut, hornbeam, birch forest, which is located to the right side of the road from the direction of Bosanska Krupa to Bužim. On the chosen location, one was able to observe the remains of wheat harvest, and as stated previously, during the basic tillage process prior to wheat sowing, about 10 t of manure per ha was used. The results of the analysis are shown in Table 7.

Table 7. Chemical properties of the soil, site Baštra

Use	Horizon	Depth in cm	Content measured in organic matter %	pH		P_2O_5	K_2O
				H_2O	1MKCl	mg/100g soil	
Natural soil	A	0 – 28	2,8	5,0	3,7	0,7	13,5
	Bv	28 – 69	0,5	5,1	3,8	0,2	8,6
	BvC	69 - 100	0,2	5,0	3,7	0,4	7,7
Arable soil	Ap	0 – 20	1,9	6,2	5,7	0,8	10,8
	Bv	20 – 43	0,4	6,1	5,3	0,5	6,9
	BvC	43 - 100	0,1	5,0	3,6	0,4	9,6

The pH in 1M KCl, reveal itself as being extremely acidic in the natural soil, while pH values in the anthropogenic soil show a moderate acidity. Likewise, the pH of the H_2O in anthropogenic soil ranges from 5,7 to 6,2 within the Ap-horizon and as the depth increases so does the acidity; while the pH values in H_2O measured in the natural soil are much lower, thus it ranges in the A-horizon from 3,7 to 5,0, and the depth does not change. The humus content in the non-arable pastures can be characterized as moderately humified (2,8%), while the anthropogenic soil has been revealed to be poorly humified (1,9%). This phenomenon can be considered as a negative byproduct of anthropogenization, during which a loss of humus in the anthropogenic soil has occurred. The content of physiologically active phosphorus in both profiles is at 1mg per 100 g of soil throughout every horizon, and it can thus be concluded that a shortage in phosphorus content within the soil has occurred. The potassium values vary very low, although the natural soil shows

a slightly higher content of potassium than the anthropogenic soil. Potassium values are highest in the topsoil, decreasing in the other horizons, but it can generally be said that the medium layer of the soil is well endowed with potassium. The results of the analysis are shown in Table 8.

Table 8. Chemical properties of the absorption complex according to Kappen, site Baštra

Use	Depth in cm	Hydrolytic acidity (H)	Content of exchangeable bases (S)	Total absorption capacity (T)	Degree of base saturation related to Total absorption capacity (V)
		cmol/kg^{-1}	cmol/kg^{-1}	cmol/kg^{-1}	%
Natural soil	0 - 10	11,3	11,5	22,9	50,3
	10 - 20	9,7	7,9	17,6	44,9
Arable soil	0 - 10	3,2	20,3	23,5	86,2
	10 - 20	3,2	18,3	21,5	84,9

The degree of base saturation in the anthropogenic soil was measured 86,2% at the depth of 0 to 10 cm, and 84,9% at the depth of 10 to 20 cm. When it comes to natural soil, values are different, so the degree of base saturation at a depth of 0 to10 cm was 50,3% and 44,9% at the depth of 10 to 20 cm. These differences are a reaction to twice as large values measured within the exchangeable bases. Higher values measured in the degree of base saturation, higher pH values, and a lower humus content observed in the soil on the arable land, can be explained by the location where the soil profile was sampled, namely at the bottom of the slope, where bases,washed down from higher parts of the slopes, had accumulated.

CONCLUSIONS

The land used for gardening in general, from where samples for testing were taken, is characterized by fertilization with high doses of organic fertilizers, and as the tests confirm, the impact of anthropogenization on these soils can be considered positive. It is also important to point out that the content of phosphorus and potassium, as well as pH values is higher in the anthropogenic soil, than in the natural soil. And finally, it is important to mention that no increase in the content of heavy metals was observed in the sampled and tested soil profiles.

Thus, all this can be characterized as favorable anthropogenic activities which occurred on the sampled and tested soil.

Observing it from the agro-ecological standpoint, whilst taking most of the field study parameters and the above listed data into account, it can be concluded that the anthropogenic soil is a convenient medium for the cultivation of most field and vegetable crops. In order to improve the fertility and productivity of natural soils, which have been tested, certain measures are recommended; namely pedo-meliorative calcification with humization steps in order to enhance the quality of the soil. A byproduct of this measure would be a reduction in the acidity, toxic effects of Al^{3+} ions (as a symptom of a very strong acidity), chemical immobilization of phosphates, and lastly, an increase of the Ca^{2+} ion in the absorptive complex of the soil. By maintaining the afore mentioned measures, along with regular fertilization on the basis of a strong P-formulation, implementing the use of KAN and other regular agro-measures, and lastly, planting the seed of a high genetic potential, one would achieve a higher crop yields on these plots of land.

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ANTI-INFECTIVE AND ANTI-TUMOR ACTIVITY OF SOME METAL COMPLEXES (M^{II}-M^{IV}) WITH SCHIFF BASES

PROFESSIONAL PAPER

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ABSTRACT

Research and application of metal complexes of ruthenium, platinum, palladium and other d-block elements has been popular in recent time because the complexes of said metals with a wide range of organic ligands shown to be extremely efficient in the treatment of infectious and malignant diseases. In addition to platinum used long time as Cisplatin, Carboplatin and oxaplatin, new generation of anticancer complexes in their structure containe mainly Ru(II) and Ru(III). In the synthesis of anti-infective and anti-tumor drugs, emphasis is indicated on their cytotoxicity. Specifically, the aim is that the new anti-infective and anti-tumor agent does not damage healthy cells and affects only malignant cells or infectious agents.
In this paper we make reference on some recent and significant researches in the field of inorganic synthesis of metal complexes with strong anti-tumor and anti-infective properties. Special emphasis is placed on the Schiff bases as organic ligands which are specially used in the synthesis of such agents.

Keywords: metal complex, antimicrobial activity, organic ligands, in vitro

INTRODUCTION

Schiff bases are formed when any primary amine reacts with an aldehyde or a ketone under specific conditions[1].The general reaction of forming Schiff base is shown in Figure 1.The stability of Schiff base varies depending on the substituents on the amine or carbonyl group. Some Schiff bases undergo to hydrolyse, so it is necessary to continuously remove water that is formed during the reaction[2]. It is observed that Schiff bases from the aldehydes are formed much easier than Schiff bases from the corresponding ketones, which is to be expected due to the higher reactivity of aldehydes compared to ketones, and due to their lower steric hindrance. It is also known that Schiff bases with an aromatic substituents, as in the carbonyl and in the amine, are much stable[2].
One of the interesting ability of Schiff bases is their ability to act as ligands of coordination compounds (mono-, di-, tri-, and tetradentate), followed by deprotonation
of the Schiff base. These compounds are usually prepared by coordination with a metal acetates which easily deprotonate imines forming acetic acid[2,3].Biological activities of Schiff bases is reflected in the antibacterial, antifungal and antitumor activity. Some transition metal complexes of Schiff's bases,whichare biologically active and have antibacterial activity, are used as radiopharmaceutical agent, system models of biological macromolecules and as transporters of molecular oxygen[4].

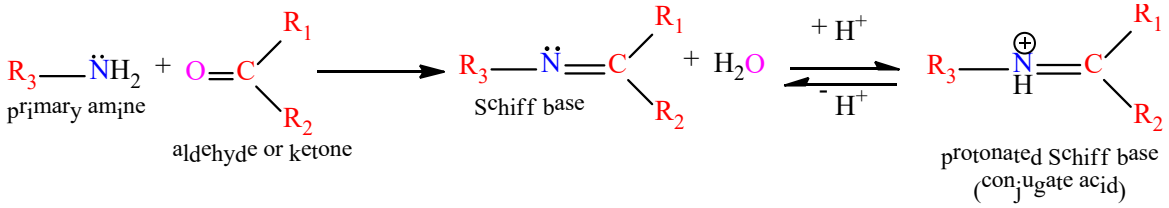

Figure 1. The general reaction of forming Schiff base

Complex with anti-infective properties

Metal complexes of Schiff base derived from 2-thiophene carboxaldehyde and 2-aminobenzoic acid (HL) and Fe(III) or Co(II) or Ni(II) or UO_2(II) showed good antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus pyogenes*. Fe(III), Cu(II), Zn(II) and UO_2(II) complexes caused inhibition for E. coli. The importance of this lies in the fact that these complexes could be applied fairly in the treatment of some common diseases caused by *E. coli*. However, Fe(III), Co(II), Cu(II), Zn(II) and UO_2(II) complexes were specialized in inhibiting Gram-positive bacterial strains (*Staphylococcus pyogenes* and *P. aeruginosa*)[1].Four Platinum(II) Schiff bases complexes containing of salicylaldehyde and 2-furaldehyde with o- and p-phenylenediamine were reported as antibacterial against *E. coli*, *Bacillus subtilis*, *P. aeruginosa*, *Staphylococcus aureus*. The activity data show that the platinum(II) complexes are more potent antimicrobials than the parent Schiff base ligands against one or more microorganisms[1,5].

Antimicrobial properties exhibit complexes M(II) with Schiff bases derived from cefotaxime with 1H-indole-2,3-dione (isatin) and 4-N, N-dimethyl amino-benzaldehyde. Synthetic scheme of two Schiff bases derived cefotaxime is shown in Figure 2.[6].

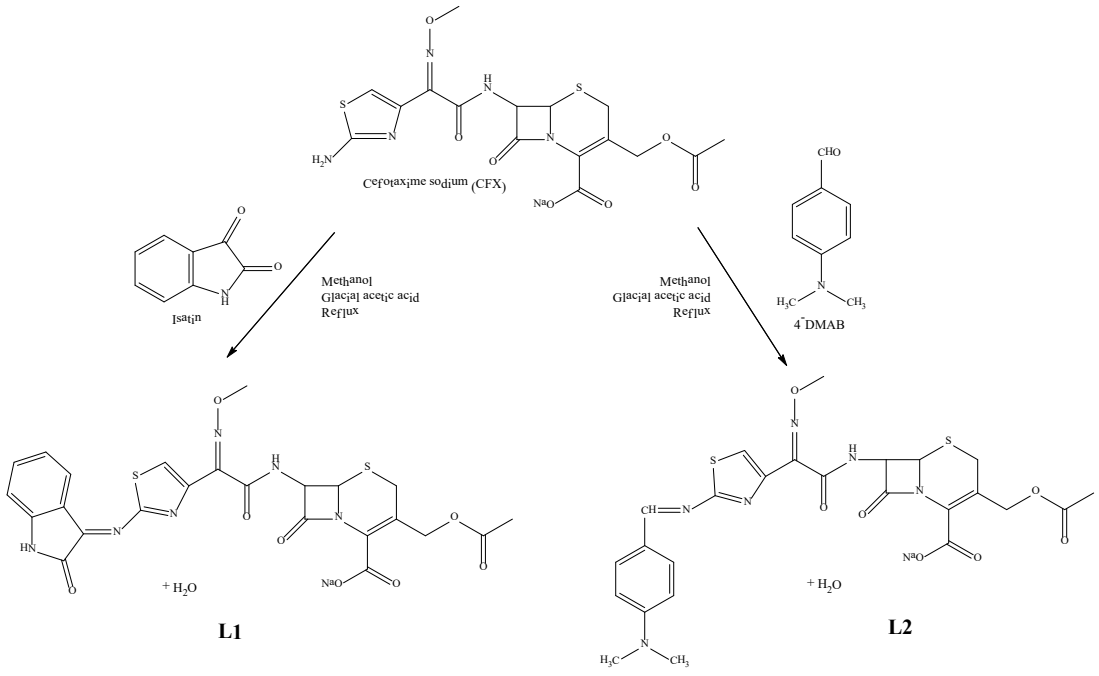

Figure 2. Synthetic route of the two Schiff base derivatives of cefotaxim

As a part of this study, synthesis of complexes Co(II), Ni(II), Cu(II), Cd(II), $PdCl_2(PhCN)_2$] and K_2PtCl_6 with the obtained ligands were performed. By subjecting the resulting products of thermal elemental analysis, NMR and FT-IR spectroscopy was found to be a tridentate ligand and that all the complexes have an octahedral geometry, except square planar structureof Pd(II) complex. Investigation of antimicrobial activity of complex prepared in DMSO (1 mg/ml) is performed in vitro on *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus pneumoniae*, using the agar diffusion technique.Main ligands have proved to be completely inactive in all research culture at the selected concentration, indicating that the original amino group of antibiotic has an important role in inhibiting the growth of bacterial cultures. All metal complexes showed selective activity against one or two bacterial species, other than the complex [Pt(LII)Cl_3] $H_2O \cdot 0.5$ EtOH which acted against all culture and showed greater activity against *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*, compared to free antibiotics (Cefotaxime). Other complexes have proven to be moderately active against *Staphylococcus aureus*[6].

Complexes of some transition metals with certain Schiff bases derived from o-phenylenediamine and 5-nitrosalicyl-aldehyde were subjected to standard antimicrobial assay against *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*[7]. Synthesis of Schiff base used in the following study is shown in Figure 3.

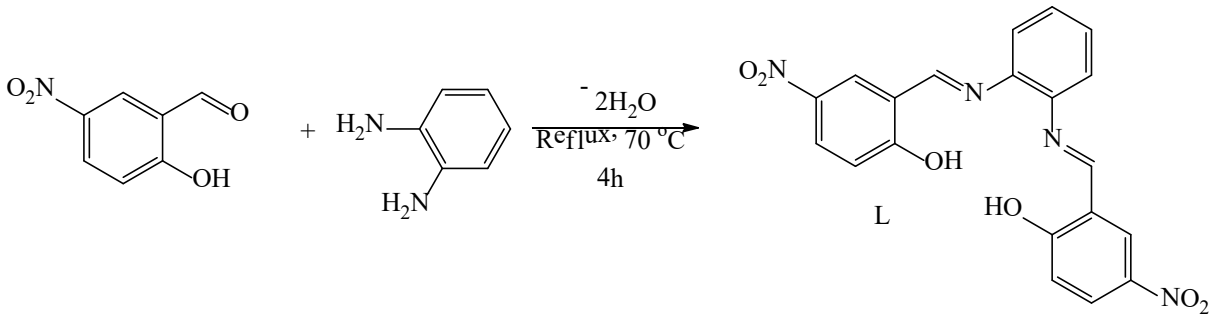

Figure 3. Synthesis of Schiff base from o-phenylenediamine and 5-nitrosalicylaldehyde

For synthesis of complexes were used Cu(II), Ni(II), Co(II) and Fe(III). Complex concentrations for investigations of antimicrobial properties against *E. coli*, *S. typhi*, *S. aureus* and *P. aeruginosa* were 5 mg/mL. Table 1 presents the obtained results[7].

Table 1. Antimicrobial activity of Schiff base as ligand and synthesized complexes

Diameter of inhibition zone of bacteria (mm)				
Compound	*S.typhi*	**S.aureus**	*P. aeruginosa*	*E.coli*
L	12	12	-	16
Fe-L	5	-	-	10
Co-L	12	16	-	8
Ni-L	8	10	-	8
Cu-L	10	10	-	11

From the table it can be clearly concluded that a Schiff base ligand having pronounced antimicrobial properties in comparison to the synthesized complex M-L. The researchers found that thegeometry of formed complexes is important for their antimicrobial activity. Namely, a square planar geometry of complex (in the case of Ni(II) and Cu(II)) showing lower activity against all strains of the bacteria compared to the parent ligand. Octahedral cobalt complex shows high activity against Gram-positive *S. typhi* and *S. aureus*[7].

Antimicrobial and antifungal study of complexes of Zn(II) with Schiff bases was made by scientists in India. After synthesis of two Schiff base, IR spectroscopic characterization is carried of the same and their proposed structure is shown at Figure 4.[8] After synthesis of ligand, complexes ZnL and ZnL_2 has been synthesized, and their proposed structures are shown in Figure 5.

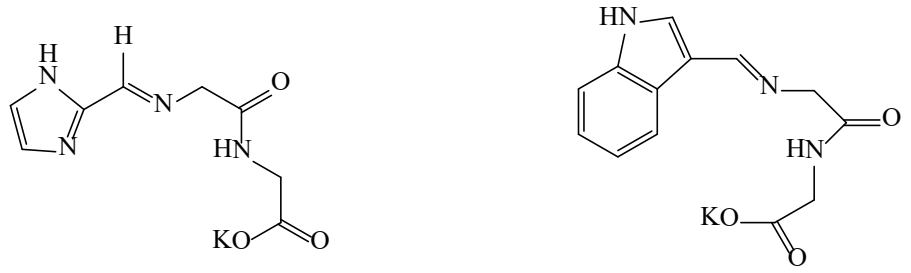

Figure 4. Proposed structure of two Schiff bases

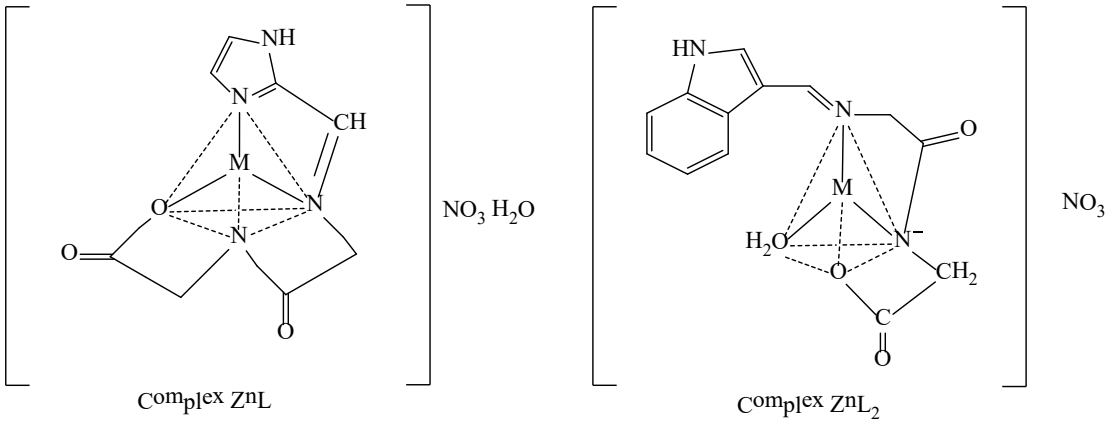

Figure 5. Proposed structure of complexes ZnL and ZnL_2

Antimicrobial activity *in vitro* of ligand and synthesized complexes were tested on *Staphylococcusaureus, Escherichia coli, Klebsiella pneumaniae, Proteus vulgaris* and *Pseudomonas aeruginosa*, whereas the antifungal activity was tested on *Aspergillus niger, Rhizopus stolonifer, Aspergillus flavus*, *Rhizoctonia bataicola* and *Candida albicans*. The study showed that both compounds showed a high activity in bacterial strains of *S. aureus, E. coli* and *P. aeruginosa*. In case of *Klebsiella pneumaniae*, ZnL complex indicates moderate activity, while the other complex (ZnL_2) is significantly more active. As for the antifungal activity of both complexes, for ZnL was found to have a higher activity of *A. niger*, *R. stolonifera* and *A. flavus*. ZnL_2 complex shows a higher activity of *A. niger, A. flavus and C. albicans* in comparison to *R. Stolonifer* and *R. bataicola*[8].

Complex with anti-tumor properties

Due to the increase of malignant diseases in the world, a great emphasis is placed on the synthesis of new inorganic, antitumorcomplexes. In the following it will be described some interesting papers in this area.

Anti-tumor activity of complexes Pt(IV), Au(III) and Pd(II) with Schiff base derived from 2-furaldehyde and 4-aminoantipyrine were performed by the scientists from University Princess Nora Bint Abdul Rahman. The structure of Schiff base (4 -[(furan-2-ylmethylene) -amino] -1,5-dimethyl-2-phenyl-1,2-dihydro-pyrazol-3-one, short 4APF) and the proposed structure of formed complexes are shown at Figure 6.[9].

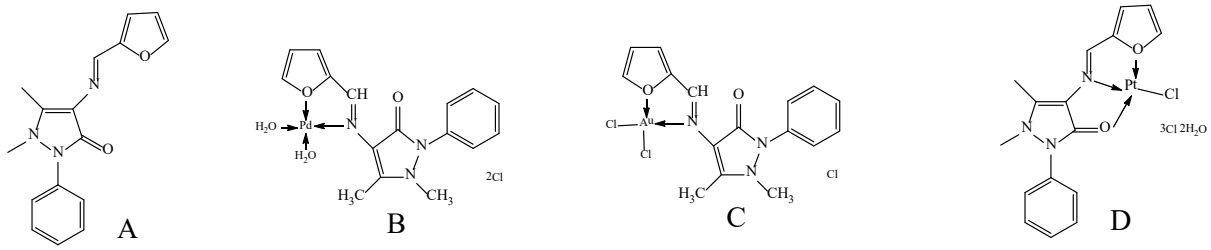

Figure 6. Structure of Schiff base derived from 2-furaldehyde and 4-aminoantipyrine and the proposed structure of synthesized complexes: A) 4 - [(furan-2-ylmethylene) -amino]-1,5-dimethyl-2-phenyl-1,2 dihydro-pyrazol-3-one (4APF); B) $[Pd(4APF)]Cl_2$; C) $[Au(4APF)(Cl)_2]Cl$; D) $[Pt(4APF)Cl]Cl_3$

Antitumor activity was tested on breast cancer cells (MCF-7 cell line). The results showed significant anti-tumor activity of the complex synthesized in said cell lines. Different concentrations of complexes (50; 25; 12,5; 6,25; 3,125 and 1,56 mg) were used and the cell viability (%) was determined by colorimetric method. The inhibitory concentration for parent ligand was 3,2 mg, 4,7 mg of Pt(IV) complex, 3,82 mg of Au(III) and 18,6 mg of the Pd(II). This paper suggests clinically achievable concentrations of 4-APF Schiff bases which may be useful in the destruction of the MCF-7 breast cancer cell lines[9]. Since platinum is one of the main metal used for the synthesis of anticancer complexes, considerable research is based precisely on the application of this metal. A group of scientists investigated antitumor activity of Pt-complex and have published their work in the Canadian Journal of Chemistry. Scheme of the synthesis of seven new (salicilaldiminato) platinum (II) complexes is shown in Figure 7, and their antitumor activities were examined.[10].

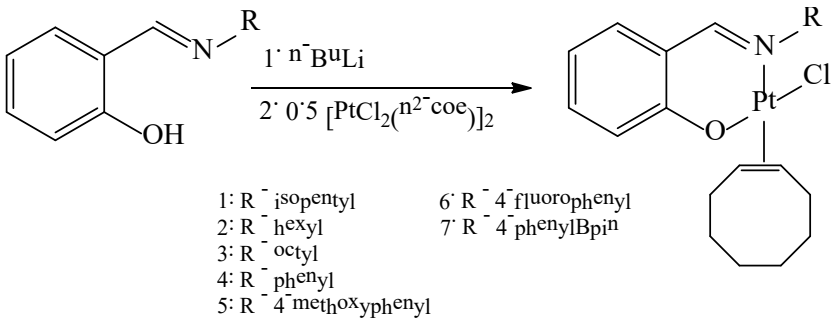

Figure 7. The synthesis of seven new (salicilaldiminato) platinum(II) complexes

Antitumor activity of synthesized complexes were subjected i*n vitro* to aggressive breast cancer (cell line MB231) and RCC cell lines. Complex indicated as 7 proved to be unstable, so antitumor activity was examined for the remaining complexes. Complex 3, which includes the longest aliphatic chain (R-octyl group) proved to be promising in inducing apoptosis of MB231 cell lines. On the other hand, complexes 4-6 (R-aromatic group) showed significant cytotoxicity in RCC cell lines. However, a disadvantage of these complexes is to cause damage to healthy cells which is not desired in the treatment of oncological diseases[10].

Complexes of ruthenium have great potential for use in cancer therapy and is the only non-platinum complexes which have entered the phase of clinical testing as chemotherapeutics. Rutheniumcomplexes have at least three significant characteristics, which is considered that could be successfully applied in therapy: kinetics of ligand exchange, a wide range of oxidation states and rutheniumproperty to imitate iron in biochemical processes[11].

Today, a large number of Ru(II) and Ru(III) complexeswith a wide spectrum of organic ligands are investigated. One of interesting research is performed by scientists at the Faculty of Natural Sciences and Agriculture, University of Fort Hare in South Africa. Specifically, they examined antitumor activity of Ru(III) complexes with tridentate Schiff bases. They examined anti-tumor potential of complex in MCF-7 breast cancer cell lines, renal TK-10 cell lines, and UACC-62 melanoma cell lines[12]. Synthesis of Ru(III) complex is shown in Figure 8.Results i*n vitro* studies of anticancer properties of complex which structure is shown in Figure 8, are presented in Table 2.[12]

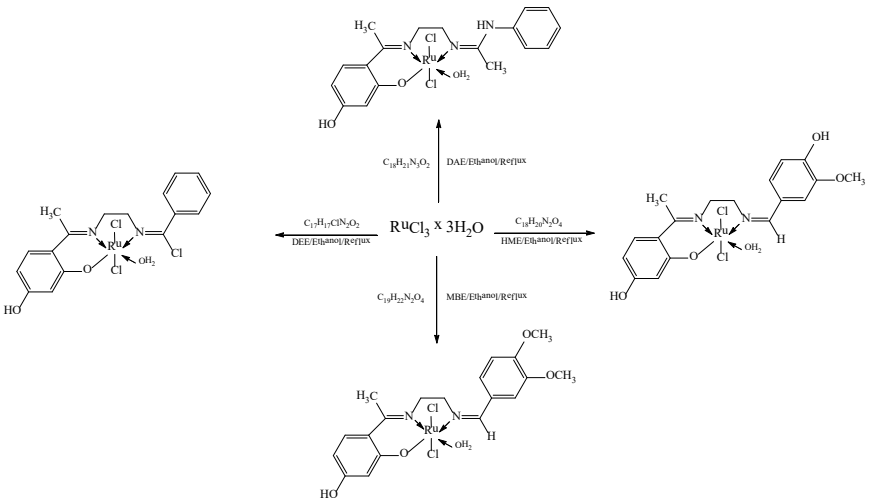

Figure 8. Synthesis of Ru(III) complexes with tridentate Schiff bases

Table 2. *In vitro* antitumor activity of Ru(III)-Schiff base complex against TK-10, UACC-62 and MCF-7 cell lines

Compounds	Molecular formula	Anticancer activity IC_{50} (μM) 48 h		
		TK-10	UACC-62	MCF-7
$[Ru(DAE)Cl_2(H_2O)$	$C_{18}H_{24}N_3O_4RuCl_2$	9,06 ± 1,18	6,44 ± 0,38	3,57 ± 1,09
$Ru(HME)Cl_2(H_2O)$	$C_{18}H_{23}N_2O_6RuCl_2$	41,09 ± 4,44	6,31 ± 1,47	4,88 ± 1,28
$Ru(DEE)Cl_2(H_2O)$	$C_{17}H_{20}N_2O_4RuCl_3$	13,10 ± 2,81	5,14 ± 1,09	3,43 ± 1,48
Parthenolide*	$C_{15}H_{20}O_3$	0,50 ± 1,43	0,89 ± 2,18	0,44 ± 2,02

* Standard cytotoxic drug

The cell lines were treated with various concentrations of complex in order to achieve 50% inhibition during 48 hours. The order of complexactivity against UACC-62 is: [Ru(DEE) $Cl_2(H_2O)$] > [Ru (HME)$Cl_2(H_2O)$] > [Ru(DAE) $Cl_2(H_2O)$]. In the case of activity of anti-TK-10 cell lines, based on the table data, it is clearly contemplated that the maximum activity shows [Ru(DAE)$Cl_2(H_2O)$ and the lowest Ru(HME) $Cl_2(H_2O)$. Based on previous reports, compounds which have an IC_{50} in the range of 10 to 25 μM have been characterized as a drug with poor antitumor activity, and those compounds whose IC_{50} in the range 5-10 μM are agents with a moderate antitumor activity. Compounds having an IC_{50} less than 5 μM are classified in strong anti-tumor agents[13]. In comparison to the research Ru(III) complexes of other scientists, these complexes have proved to be antitumor agents of high activity[12].

CONCLUSION

Based on some results presented in the field of chemistry of complex compounds, compounds with anti-tumor and anti-infective properties are today topics in interest of many scientists. The results show that there is a wide range of organic ligands in the form of complexes with a specified metal ions (such as Ru(III), Pt(IV), etc.) show the excellent efficacy in the treatment of malignant and infectious diseases. However, it still has not found an adequate way that will reduce the negative impact of these compounds on healthy cells, which certainly should be subject of additional interest and interpretation in the next period.

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EFFECTS OF BARLEY FLOUR ADDITION AND BAKING TEMPERATURE ON B-GLUCANS CONTENT AND BISCUITS PROPERTIES

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

The aim of this study was to investigate opportunities to improve the nutritional value of biscuits. Therefore, the content of β-glucans, physical, chemical and sensory properties of biscuits were determined in relation to a share of added barley flour and a baking temperature. Five different blends of barley and wheat were used for biscuit production: barley/wheat flours in combinations: 0/100; 25/75; 50/50; 75/25 and 100/0 according to the procedure described in AACC method 10-52. The temperatures used for baking were 150 and 205°C for 15 and 11 min, respectively. The obtained results showed the higher β-glucans content in samples when share of barley flour in biscuit formula was higher. The same trend was found on both baking temperatures. Besides, the share of barley flour in samples significantly influenced physical, chemical and sensory parameters. In addition, different baking temperatures affected significant differences between samples according to all parameters investigated, except N (%) and ash (%). After 6 months, β-glucan content was significantly lower in samples with high share of barley flour (75 and 100%), at both baking temperatures.

Key words: β-glucans, barley biscuits, baking temperature, sensory properties.

INTRODUCTION

Barley (*Hordeum sativum* L.) has a long history in food production. In the ancient world barley was one of the most important food grains, grown mostly to provide food resource for human nutrition. As alternative food grains, especially wheat, became available, consumption of barley decreased[1]. However, interest in barley as a food grain is currently reviving and there is an increase in new food products with barley, including biscuits, mainly due to the content of health promoting components[2,3]. Barley is mostly known for its high amount of dietary fibre, but it also has a high content of phenolic acids and other phenolic compound[2,4]. β-glucans, considered as the major fibre constituents of barley, have been implicated in several health benefits[5,6,7,8].

Mixed linkage (1→3)(1→4)-β-D-glucans, commonly named as β-glucans (BG), are linear homopolysaccharides composed solely of D-glucopyranosyl (Glcp) residues linked mostly via two or three consecutive β-(1→4)- linkages which are separated by a single β-(1→3)-linkage[3,9,10]. These polysaccharides are the major component of endosperm and aleurone layer cell walls of commercially important cereals, such as oat, barley, wheat, rye, sorghum and rice[10,11,12,13]. Barley grain is a rich source of BG[3,14,15], which are more concentrated in the endosperm[12]. The content of BG in barley according to the literature references is shown in Table 1.

Barley BG have a positive impact on human health. Barley contribute to lowering the plasma cholesterol, improving lipid metabolism, reducing the glycemic index and reducing the risk of colon cancer[5,6,7,8].

Currently, barley is increasingly incorporated in already established and new food products either as a whole grain or as a food ingredient[2]. The application of barley in these products contributes to an increased content of total and soluble fibre and improved physiological efficacy of fibres[14]. So far, various forms of barley (β-glucan-rich fractions from barley or purified β-glucans) have been added to food products, such as cereals, pasta, noodles, and various baked products (bread, cookies, muffins)[6,16,17,18,19,20,21,22,23] as well as dairy and meat products[24,25]. The nutritional value of food with added barley depends on the amount and type of barley added (hull-less or hulled)[26].

Table 1. Content of BG in barley according to the literature data

BG content (g/100 g of dry mass)	Reference
2,41–8,25	Holtekjolen et al.[13]
1,86–5,37	Havrlentová and Kraic[36]
3,24–4,62	Zhang et al.[15]
6,0–8,0	Lambo et al.[37]
3,75-7,96	Gajdošová et al.[38]
3,20–4,60	Demirbas[11]

Along with providing physiological benefits, the incorporation of different forms of barley changes processing parameters and handling, as well as food texture and some sensory properties, like a delicate nutty flavour. In addition, the incorporation of cereal BG, such as barley BG, improves the stability during storage[27].

Thus, the barley enriched products have potential to exhibit acceptable sensory properties, especially with incorporation of barley ingredients at low to moderate levels. Berglund et al.[16] reported that sensory scores of various wheat-based products including bread, bars, muffins and cookies prepared with barley added in different share were similar to those for the standard products. Similar results were reported by Newman et al.[22] for baked products, such as bread, biscuits, sugar cookies and muffins enriched with the barley fibre and by Knuckles et al.[21] for bread with 20% β-glucans barley fractions. Sudha et al.[23] found that biscuits containing 20% barley bran were highly acceptable. Gupta et al.[18] showed that barley flour incorporation improved the colour of the cookies, where the level of 30% was the best. The same level of incorporation was reported by Hassan et al.[19] as the best in terms of overall acceptability of biscuits enriched with barley meal, and as the barley meal ratio increased, all sensory attributes scores also increased.

Biscuits are popular baked products popular as food product due to their favourable taste and texture. These products are available in a variety of tastes, good nutritional quality, affordable cost and long shelf life[28]. These products have been suggested as a good way to use composite flours with the aim of improving nutritional value, since they are ready-to-eat, provide a good source of energy, and are consumed widely throughout the world[29].

This study was undertaken to investigate the opportunities to improve the nutritional value of biscuits by preparing biscuits with different combinations of wheat and barley flour. In addition, the study aimed to evaluate the effects of barley flour and baking temperature primarily on BG content and physico-chemical and sensory characteristics of biscuits.

MATERIALS AND METHODS

Materials

Barley and wheat flour samples of milling extraction rate of 87%, provided by local farmers from Breza and Nišići, Bosnia and Herzegovina, were used in this study. Blends of wheat and barley flour were obtained by replacing wheat flour with barley flour at 0%, 25%, 50%, 75% and 100%.

Blends were analyzed for water (WAC) and oil absorption capacity (OAC) as described by Sosulski et al.[30]; bulk density (BD) and swelling capacity (SC) according to Okaka et al.[31].

Biscuit-making procedure

Biscuits were prepared according to the procedure described in AACC method 10-52[32] with some

modifications. Sucrose level was reduced from 60% for the standard recipe to 40%, and 5 cm diameter circular mould was using for cutting, instead the 6 cm one, required by method. The amount of used flour was calculated based on 14% moisture content. The dough was sheeted to a thickness of 8 mm, cut using a circular mould and baked at different temperatures (150 and 205°C) for different times (15 and 11 min). After baking, biscuits were cooled for 30 min. A part of produced biscuit samples was packed in sealed bags, stored for 6 months (20°C±2, 40±5% humidity) and used for BG determination. Total of 10 biscuit samples were prepared in two replicates.

Physical analysis of biscuit samples

Diameter and thickness before and after baking were measured with an electronic digital vernier caliper (MIB Messzeuge GmbH, Spangenberg, Germany) with a sensitivity of 0,01 mm. Diameter of biscuits was determined by taking an average value of diameter of five biscuits from each replication; simultaneously, thickness was determined by taking an average of thickness of five biscuits from each replication. Consequently, the average of ten biscuits was recorded for each sample. Diameter (DI) and thickness increase (TI) were calculated as the ratio of biscuits diameter and thickness before and after baking, and expressed in %. Spread ratio (SR) was calculated by dividing the average values of diameter by thickness for 10 biscuits at a time. Specific volume (SV) was calculated as volume (seed replacement) divided by biscuits weight.

Chemical analysis

The BG content in flour blends, biscuit samples and samples after 6 months was determined according to the enzymatic-gravimetric method (AOAC method 995.16)[33] using the Mixed-linkage beta-glucan assay kit (Megazyme, Bray, Ireland). Total nitrogen (Kjeldahl) and ash contents were determined using the standard methods of analysis[33].

Sensory evaluation

The sensory evaluation was carried out by a panel of 10 trained members at the Faculty of Agriculture and Food Sciences, University of Sarajevo, B&H. The panelists were selected and trained according to recommendations in ISO 8586[34]. They participated in five 1h trainings and three 30 min evaluation sessions on different days over 4 weeks. Prior to the assessment, the panel members were trained on various samples of biscuits, made in different combinations of barley and wheat flours. The biscuits were evaluated by Quantitative Descriptive Analysis (QDA) using a scale of 1-5 scores on 4 properties: taste, aroma, melting and overall acceptability. The obtained sensory data were counted from 10 replicates (panelists were considered as replicate).

Statistical analysis

All results are expressed as mean ± standard deviation (SD). One-way analysis of variance was applied on flour blends properties; and differences between BG content in samples immediately after baking and 6 months of storage. Two-way analysis of variance with interactions (ANOVA) was used to evaluate whether significant differences existed between the biscuit samples depending on barley flour addition and the baking temperature. Determined differences were tested by the Tukey test for $p < 0,05$. Multivariate analysis of data by Principal Component Analysis (PCA) using the statistical computer package StatBox 6.7 (Grimmer Soft, France) was performed to obtain comprehensive visualization of the samples relationships.

RESULTS AND DISCUSSION

Flour blends characteristics

The results of blends characteristics (Table 2) showed an increase of WAC from 1,35 to 2,10 fold ($p < 0,05$) and SC from 20,25 to 22,25 mL while the BF share in blend was higher. Significant differences between flour blends in terms of OAC and BD were found, also.

Table 2. Effects of barley flour addition on physical blends characteristics

Barley flour (%)	WAC (%)	OAC (%)	BD (g/mL)	SC (mL)
0	1,35 ± 0,21c	1,50 ± 0,00c	0,72 ± 0,01b	20,25 ± 0,35a
25	1,40 ± 0,14c	1,90 ± 0,00ab	0,74 ± 0,01a	21,00 ± 0,71a
50	1,50 ± 0,00bc	1,85 ± 0,07b	0,72 ± 0,00ab	21,50 ± 0,71a
75	1,95 ± 0,07ab	1,80 ± 0,00b	0,71 ± 0,00b	22,00 ± 0,00a
100	2,10 ± 0,00a	2,05 ± 0,07a	0,63 ± 0,00c	22,25 ± 0,35a

All data are the mean value ± DS of three replicates. Different letters in columns from a to c for each parameter indicate significantly different values among flour blends at $P < 0,05$.
Abbreviations: WAC, water absorption capacity; OAC, oil absorption capacity; BD, bulk density; SC, swelling capacity.

Physical and chemical characteristics of biscuits

The results of physical and chemical characteristics of biscuit samples are presented in Table 3. The physical characteristics of biscuit samples were affected significantly with addition of BF and with the baking temperature. DI and TI ranged from 38,26 to 67,38%, and -13,28 to 39,33%, respectively. The sample B0T205 had the highest, while the sample B100T205 had the lowest DI and was significantly different from other samples baked at 205°C. TI of some samples was decreased during baking at both baking temperatures (B100T150 and B0T205) which indicated a negative value of TI. As the level of added BF samples baked at 205°C was increased, TI was significantly increased, while DI was significantly decreased. The changes in DI and TI were consequently reflected in SR values. SR values were decreased while BF addition in blends was higher, but this trend was found only when the baking temperature was 205°C. The similar decreasing trend of SR values were reported by Gupta et al.[18]. SV ranged from 1,52 to 2,39 cm3/g. The sample with no BF added (B0T205) had the highest SV as similar to the results reported by Osella et al.[35].

Table 3. Physical and chemical characteristics of biscuit samples in relation to BF addition and baking temperature

T	**150°C**						**205°C**								
M	**B0**	**B25**	**B50**	**B75**	**B100**	***T***	**B0**	**B25**	**B50**	**B75**	**B100**	***T***	***T***	**M**	***T*xM**
DI (%)	64,12 ± 8,20a	61,42 ± 3,80a	47,03 ± 5,36b	49,55 ± 6,52b	58,98 ± 15,17a	x	67,38 ± 6,04a	63,84 ± 4,98ab	58,63 ± 6,81bc	51,98 ± 3,83c	38,26 ± 4,08d	y	***	***	***
TI (%)	3,64 ± 4,91c	5,94 ± 8,08b	7,43 ± 6,12b	15,79 ± 11,93a	–13,28 ± 7,21d	x	–4,41 ± 5,82c	0,06 ± 4,20c	1,04 ± 5,32c	17,59 ± 8,90b	39,33 ± 7,94a	y	***	***	***
SR	9,93 ± 0,82b	9,58 ± 0,91b	8,59 ± 0,68c	8,14 ± 0,81c	11,52 ± 1,36a	x	10,99 ± 0,89a	10,26 ± 0,69ab	9,85 ± 0,83b	0,77 ± 8,13c	6,21 ± 0,25d	y	***	***	***
SV (cm^3/g)	1,58 ± 0,11a	1,54 ± 0,20a	1,61 ± 0,35 a	1,71 ± 0,17a	1,79 ± 0,26a	x	2,39 ± 0,60a	1,89 ± 0,10bc	1,98 ± 0,17ab	2,11 ± 0,26ab	1,52 ± 0,16c	y	***	***	***
Ash (%)	1,49 ± 0,01b	1,59 ± 0,04ab	1,54 ± 0,02ab	1,82 ± 0,05ab	1,89 ± 0,08a	x	1,58 ± 0,12b	1,56 ± 0,01 b	1,64 ± 0,03b	1,83 ± 0,04ab	2,03 ± 0,03a	x	ns	**	ns
Total N (%)	0,94 ± 0,01a	0,97 ± 0,04a	1,01 ± 0,05a	1,06 ± 0,00a	1,00 ± 0,26a	x	0,88 ± 0,02a	0,90 ± 0,17 a	0,89 ± 0,09a	1,06 ± 0,04a	1,06 ± 0,02a	x	ns	ns	ns

Different letters in rows from a to d for each parameter indicate significantly different values among mixture at $P < 0,05$. Different letters in rows from x to y for each parameter indicate significantly different values among temperatures at $P < 0,05$.

Abbreviations: B0, B25, B50, B75, B100 – samples with 0, 25, 50, 75 and 100% barley flour added. *T* – temperature; M – mixture; TxM – interaction between temperature and mixture. DI – diameter increase; TI – thickness increase; SR – spread ratio; SV – specific volume. ns – not significant; * – significant differences at *P*-value below 0,05; ** – significant differences at *P*-value below 0,01; *** – significant differences at *P*-value below 0,001.

Ash content and N (%) were not affected significantly with the baking temperature, while WF addition affected only the ash content. The results of the ash content in samples with 25% of BF were in accordance with the results reported by Hassan et al.[19] but lower than those reported by Gupta et al.[18].

BG content in flour blends (Fig. 1A) significantly increased with the BF level added, as expected. The rising trend of the BG content in the blends is obviously related to the BG content in the biscuit after baking at both baking temperatures (Fig. 1B, C). BF content at all levels as well as all chosen baking temperatures resulted in significant influence on the BG content. Higher values of BG were found in the biscuits baked at 205°C. BG values in biscuit samples with 25% BF were similar as reported by Hassan et al.[19], but lower than those reported by Gupta et al.[18].

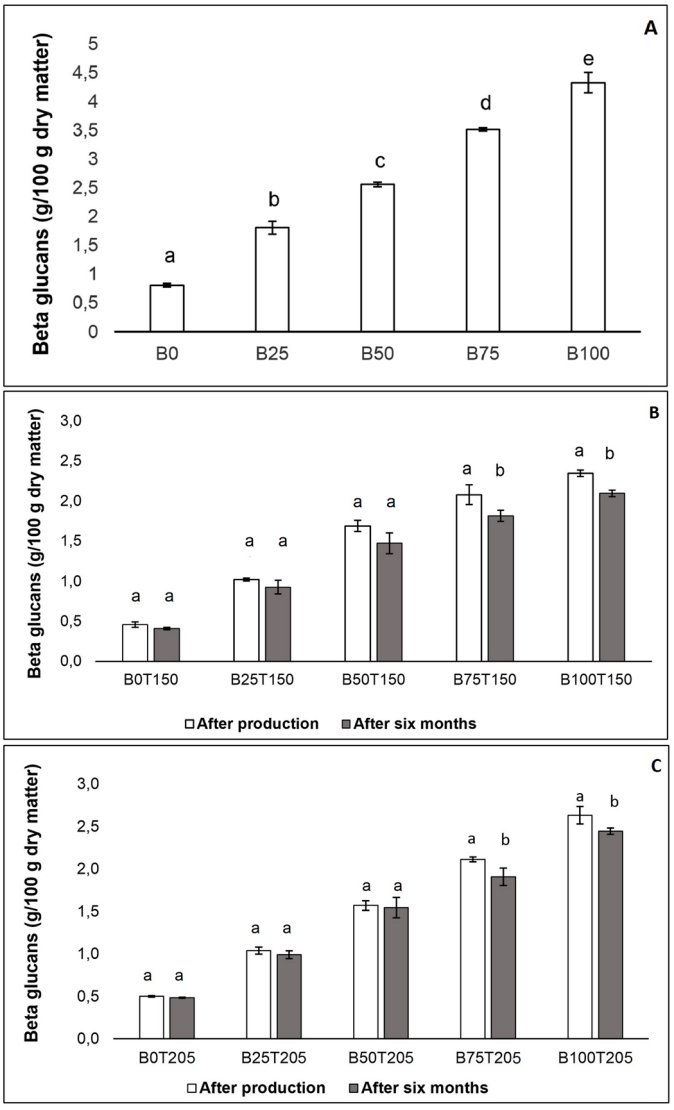

Figure 1. BG content in flour blends (A), BG content in biscuits baked on 150°C after baking and 6 months of storage (B), BG content in biscuits baked on 205°C after baking and 6 months of storage (C). Samples codes reflect share of barley in the dough B0; B25; B50, B75; B100 and used temperature of baking T 150°C and T 205°C. Different letters above the columns from a to e in each graph indicate significantly different values among flour blends (A) and the same biscuit samples (B, C) at $P < 0,05$.

After 6 months of storage, the BG content in all samples decreased, while the differences between samples containing high share of BF (75 and 100%) at both temperatures were significant.

Sensory evaluation of biscuits

According to the two-way ANOVA all sensory properties were significantly influenced by the addition of BF at all levels and the baking temperature. The samples baked at 205°C were characterized by significantly higher scores for taste, melting and overall acceptability, than the samples baked at 150°C.

Table 4. Sensory evaluation of biscuit samples in relation to BF addition and baking temperature

T	**150°C**						**205°C**								
M	**B0**	**B25**	**B50**	**B75**	**B100**	***T***	**B0**	**B25**	**B50**	**B75**	**B100**	***T***	***T***	**M**	***T*xM**
Taste	3,70 ± 0,95ab	3,65 ± 0,47a	3,75 ± 0,54a	3,85 ± 0,67a	3,35 ± 0,67a	x	3,90 ± 0,57a	3,75 ± 0,63a	3,85 ± 0,47a	4,35 ± 0,67a	3,75 ± 0,54a	y	***	***	***
Aroma	3,55 ± 1,07ab	3,80 ± 0,42ab	3,45 ± 0,69ab	4,05 ± 0,6a	3,25 ± 0,63b	x	3,70 ± 0,48ab	3,25 ± 0,42b	3,80 ± 0,42ab	4,00 ± 0,47a	3,90 ± 0,32a	y	***	***	***
Melting	3,15 ± 1,00b	3,70 ± 0,48ab	3,90 ± 0,57a	3,60 ± 0,70ab	3,85 ± 0,67a	x	3,50 ± 0,71b	3,90 ± 0,32ab	4,05 ± 0,50ab	4,50 ± 0,58a	4,15 ± 0,34ab	y	***	***	***
General acceptability	3,70 ± 0,95ab	3,80 ± 0,42ab	3,95 ± 0,60ab	4,00 ± 0,47a	3,30 ± 0,86b	x	3,70 ± 0,48b	3,80 ± 0,42ab	3,85 ± 0,47ab	4,35 ± 0,47a	4,10 ± 0,52ab	y	***	***	***

Different letters in rows from a to b for each parameter indicate significantly different values among mixture at $P < 0,05$. Different letters in rows from x to y for each parameter indicate significantly different values among temperatures at $P < 0,05$.
Abbreviations: B0, B25, B50, B75, B100 – samples with 0, 25, 50, 75 and 100% barley flour added; *T* – temperature; M – mixture; TxM – interaction between temperature and mixture. ns – not significant; * – significant differences at P-value below 0,05; ** – significant differences at P-value below 0,01; *** – significant differences at P-value below 0,001.

In general the best sensory profile was observed in the sample with 75% BF contained, baked at 205°C, as it won the highest score for taste, melting and overall acceptability. On the other hand, the sample containing only BF, baked at 150°C had the weakest sensory properties.
Sensory evaluation (taste, aroma, melting, overall acceptability) of biscuit samples revealed that in a 5-scores scale, all sensory results were in the range of 3,15-4,5 indicating that these biscuits were moderately acceptable. A kind of nutty flavour was noticed in samples with 75 and 100% BF share at the baking temperature of 205°C.

Principal Component Analysis (PCA)

The results of physical, chemical and sensory characteristics were subjected to the multivariate analysis by Principal Component Analysis (PCA) with the aim of interpreting relationships between samples concerning the BF content and the baking temperature (Fig. 2). In this analysis, those parameters which did not allow to discriminate the samples were excluded. The following variables: SR, SV, BG, ash, total N, taste, aroma, melting and overall acceptability were considered. For the here performed PCA, the two major principal components explained 79% of the data variability (Fig. 2). PC1 (56% of the total statistical significance) was correlated to the content of ash, BG and N, then SV and finally to all sensory attributes. PC2 (22% of the total statistical significance) was related to the SV and taste.

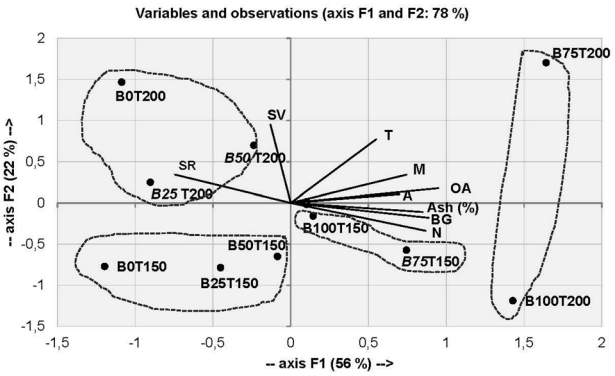

Figure 2. PCA biplot of the measured physicochemical and sensory data and overall positioning of 10 biscuit samples. Samples codes reflect share of barley in the dough B0; B25; B50, B75; B100 and used temperature of baking T 150°C and T 205°C. Physicochemical data are: SR, spread ratio; SV, specific volume; BG, β-glucans content; Ash; N. Sensory data are: T, taste; M, melting; A, aroma; OI, overall acceptability.

All samples distributed were spread on both sides of the PCA plot (related to PC1). The position of the samples was obviously dependent on the BF amount in the biscuits. The samples with a high BF content (75 and 100%) were positioned on the positive side of the plot (PC1). This side of the plot was mainly influenced by N, ash and BG. It is obvious that samples with high BF content were described by desirable sensory properties. On the other hand, the samples with no BF added and low BF content (25 and 50%) were found on the negative side of PC1. This side was mainly influenced by SR (PC1) and SV (PC2). In addition, sample clustering related to the baking temperature was observed. So, the samples with high BF amount baked at 205°C were more favourable than similar samples baked at 150°C with respect to sensory properties. Thus, higher contribution of BF means the higher content of ash, N and BG, specifically found at baking temperatures at 205°C.

On the opposite side (positive section of PC1), the samples with low BF content were located. They were mainly dependent on the baking temperature. The samples with low BF content (high WF content and baked at 205°C) were mainly described by SV and SR. Unlike this, samples baked at 150°C and containing the low level of BF were not described by any of examined parameters.

CONCLUSIONS

The here presented results confirm that barley flour is suitable addition for the biscuit formula and improves the nutrition value of the final product due to the increased BG content. In addition, barley provides acceptable sensory quality. Better physical properties and sensory quality was achieved when the baking temperature was chosen at 205°C over 150°C.

Barley flour is, thus, a good choice for improving the final baked products quality by increasing the BG content of usually used wheat flour only. However, more in-depth follow-up information of consumers is required for arguing to select these products and allow reasonable food quality assessment, especially with respect to confectionery products.

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HEAVY METALS IN WATER AND MUSCLE TISSUE OF TROUT (*Salmo trutta*) IN THE RIVER UNA

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

This paper presents the results of research on the content of heavy metals in water and fish of the River Una. During 2012 and 2013 water and fish samples were taken from four locations, and research was conducted in two seasons: autumn 2012 (I season) and spring 2013 (II season). The caught fish at all locations belonged to the species of trout (*Salmo trutta*). The content of Pb, Cu and Cd was determined by means of an atomic absorption spectrophotometer "Perkin Elmer" AAnalyst - 800, using a flame photometry technique. The content of Pb, Cu and Cd was below the level of maximum allowable concentrations (MAC). The research of heavy metal presence in the water of the River Una indicated the value of Pb lower than 0,0010 mg/l, Cu lower than 0.4 mg/l, and Cd lower than 0,1 mg/l.
Heavy metals in fish muscle tissue were directly related to the pollution of river. Lead content in muscle tissue of fish caught in the River Una was the highest in samples which were caught at the fourth location, with 0,205 mg kg^{-1}, while the highest copper content was identified in the same location, 0,1280 mg kg^{-1}. The content of cadmium in fish muscle tissue samples was below 0,1 mg kg^{-1}. The heavy metal content in the tested samples of water and fish the River Una was below the allowable limit. These results indicate that the pollution of the River Una did not reach a significant level.

Keywords: cadmium, lead, copper, fish, atomic absorption spectrophotometry

INTRODUCTION

Ecotoxic metals are metals that are "toxic" to the living world in its dissolved phase. The used term heavy metals was not entirely suitable, because some light metals, e.g. beryllium, are toxic and some heavy metals, e.g. iron, are not toxic. It is more correct to use the term metals in trace or trace metals because their natural concentrations are very low (<1 μL^{-1}), and is found in nature only in traces. However, when their concentrations raise (mostly anthropogenic), and become dangerous for the living world of the aquatic environment, the term *ecotoxic metals* is the most appropriate. Metals are an important factor in any aquatic environment since, in many cases, the biodiversity of the aquatic ecosystem depends on them. Sewerage system and waste water from industrial plants are discharged directly into the watercourse, without previous purification. Also, without separation of solid waste, electrical appliances are disposed together, so that, through residual water, heavy metals reach to brooks and rivers, which burden water and organisms of the aquatic environment. This method of waste disposal can lead to environmental exposure to harmful substances, such as, among others, heavy metals[1,2,3]

The pollution of surface water by heavy metals is a serious environmental problem especially because some of them are toxic at low concentrations, do not break down and have a cumulative effect. Although some metals, such as Fe, Cu and Zn, are essential micronutrients, they can be harmful to the physiology of living organisms in higher concentrations[4,5]. Heavy metals as pollutants are non-biodegradable, and can become toxic even when they are away from the source of pollution[6].

Heavy metals in traces, which have been extensively studied in recent decades, include Copper (Cu), Lead (Pb), Cadmium (Cd), Zinc

(Zn), Iron (Fe), Manganese (Mn), Arsenic (As), Mercury (Hg) and Selenium (Se). These elements are showing the greatest ecological interest due to frequent contamination of soil, water and food chain. Furthermore, determining the amount of heavy metals in the tissues of animals gives an insight into environmental pollution and possible immunopathological changes and intoxication of animals[7]. Environment is an essential element of human existence. This is a result of the interaction of natural elements such as earth, air, water, climate, biosphere with elements created by human activity[8]. All these interactions and effects of existential conditions and opportunities for further development of society to protect the environment, mainly contaminated areas, must be identified; evaluate the extent of damage and identify the causes that are produced by these imbalances. It is necessary to maintain the quality of the environment by reducing the negative effects of human activities. Potentially toxic metals resulting from anthropogenic activities cause serious disturbances in ecosystems[9,10].

In fish samples from a number of locations along the river course, Mazet et al.,[11] proved the presence of cadmium and lead, where their concentrations did not exceed the value defined in European Regulation (European Regulation R466/2001)[12]. The amount of lead in fish muscle tissue samples was correlated with the concentration of polychlorinated biphenyls being explained with different population density and urbanization. In the research of [13], the content of Pb in fish muscle tissue (middle value) upstream from the city of Bihać amounted to 0,29 mg/kg and downstream of the city of Bihać 0,34 mg/kg, Cu, upstream 0,82 mg/kg, downstream 1,33 mg/kg and the content of Cd upstream 1,00 mg/kg and downstream 1,64 mg/kg.

MATERIALS AND METHODS

Sampling of water and fish in the River Una was carried out in September 2012 and March 2013.

Water and fish samples of the River Una were taken for analysis at four different locations: Ripač, Orljani, Bakšaiš and Vrkašić.

Table 1. Locations of samples

Una locality	The position of locality	located (E:N)
Una – Ripač	(46,5 km from the source)	15,9534380 44,7632863
Una – Orljani	(Sunce, 55,5 km from the source) - above Bihać	15,9070863 44,8003857
Una- Bakšaiš	(60,5 km from the source) – the city of Bihać	15,8640207 44,8283788
Una-Vrkašić	(62,5 km from the source) - below Bihać - after collectors	15,8451380 44,8358962

After sampling, the water and fish samples were submitted to a portable cooling device in the laboratory, where we performed preparation of samples for analysis. The analysis was performed by atomic absorption spectrophotometry. Analyses were performed on the atomic absorption spectrophotometer "Perkin Elmer" AAnalyst - 800.

Atomic absorption spectrophotometry is an optical method based on measuring the absorption of electromagnetic rays by atoms in the ground state. The atoms in the ground state disappear by thermal dissociation[14]. In atomic absorption, concentration can be determined from measurements of atoms light absorption in the ground state during irradiation with

an appropriate excitation source. The flame emission concentration can be determined from the intensity of the radiation which is emitted at a fraction of atoms that have passed in the excited state[15]. For the most routine analysis a flame temperature around 2400°C is required, which can be achieved by, for example mixtures of air - acetylene. The sample for analysis in liquid state is introduced into the flame by spray, resulting in a dispersion of fine liquid drops[14]. The analysis performed by atomic spectroscopic methods almost always requires simple and complex sample preparation. These steps of sample preparation are generally the most critical part of the analysis, because they are responsible for most errors[16]. In order to determine heavy metals, destruction of samples (fish muscle tissue) was done by microwave digestion in the mixture of nitric acid and hydrogen peroxide, in accordance with the instructions for handling apparatus of microwave digestion (Milestone, Sart D). In 1,5 g of sample, we added 7 ml of conc. HNO_3 and 1 ml of H_2O_2, and then we burnt it at 200 ° C, on 1000 W. The first heating was carried out for 10 minutes, then 10 minutes of burning, and finally the cooling process which showed the flow diagram on display of the oven. The conditions under which the analysis of heavy metals was conducted are shown in Table 2.

Table 2. Recommended conditions for analysis on AAS

Element	Flame	Wavelength	Burner	Calibration methods	Stock Stand. Solution
Pb	Air-acetylene	283,3 nm	10 cm	Linear / zero	Lead 1000 mg/l
Cu	Air-acetylene	324,8 nm	10 cm	Linear / zero	Cooper 1000mg/l
Cd	Air-acetylene	228,8 nm	10 cm	Linear / zero	Cadmium1000mg/l

RESULTS AND DISCUSSION

To determine the pollution degree of any part of the ecosystem including water, it is necessary to determine whether pollution can harmfully affect human health or to determine the limit value of the amount of harmful substances intake, in our case of heavy metals in water and fish of the River Una, under which, based on scientific research, there is no or the least possible risk of adverse effects on human health and/or the environment as a whole. The results are presented in Table 3,4,5 and 6.

Table 3. The results of the analysis of heavy metals in water of the River Una (September 2012)

Una locality	Ripač	Orljani	Bakšaiš	Vrkašić
Pb (mg/l)	0,0000	0,0000	0,0000	< 0,001
Cu (mg/l)	< 0,4	< 0,4	< 0,4	< 0,4
Cd (mg/l)	< 0,1	< 0,1	< 0,1	< 0,1

Table 4. The results of the analysis of heavy metals in fish of the River Una (September 2012)

Una locality	Ripač	Orljani	Bakšaiš	Vrkašić
Pb (mg kg^{-1})	0,0000	0,0013	0,0000	0,2050
Cu (mg kg^{-1})	0,8001	0,9090	1,1350	0,1280
Cd (mg kg^{-1})	< 0,1	< 0,1	< 0,1	< 0,1

The research results did not exceed in any sample MAC (maximum allowable concentrations). In water research samples of both periods, the values of Pb were identified only on location Vrkašić in the concentration of less than 0,001 mg/l. In both periods, the content of copper Cu in the River Una was less than 0,4 mg/l, while the value of Cd was below 0,1 mg/l.

Table 5. The results of heavy metals in water of the River Una (March 2013)

Una locality	Ripač	Orljani	Bakšaiš	Vrkašić
Pb (mg/l)	0,000	0,000	0,000	< 0,001
Cu (mg/l)	< 0,4	< 0,4	< 0,4	< 0,4
Cd (mg/l)	< 0,1	< 0,1	< 0,1	< 0,1

Table 6. The results of heavy metals in fish of the River Una (March 2013)

Una locality	Ripač	Orljani	Bakšaiš	Vrkašić
Pb (mg kg^{-1})	0,0000	0,0000	0,0000	0,1560
Cu (mg kg^{-1})	0,4010	0,5020	0,7630	0,0650
Cd (mg kg^{-1})	< 0,1	< 0,1	< 0,1	< 0,1

Vidaček et al.,[17] explored the content of heavy metals in Karašica-Vučica water basin. Concentration values of Cu results ranged from 0,0033 to 0,0213 mg/l, Pb from 0,0046 to 0,0405 mg/l, Cd from 0,0002 to 0,0039 mg/l, being consistent to our results. According to the Regulations on maximum levels for certain contaminants in food (Official Gazette of BiH 37/09)[18], the values of heavy metals Pb, Cu and Cd in fish muscle tissue samples did not exceed allowed concentrations. In trout muscle tissue samples (*Salmo trutta*) overfished in the period of September 2012, the content of Pb at the locality of Orljani (55,5 km from the source-over Bihać) was 0,0013 mg kg^{-1}, while the value at the locality of Vrkašić (62,5 km from the source below-Bihać-after collector) was increased to 0,2050 mg kg^{-1}. The determined amount of Cu in the muscle tissue of trout (*Salmo trutta*) ranged from 0,8001 mg kg^{-1}, the locality of Ripač (46,5 km from the source) to 1,1350 mg kg^{-1}, the locality of Bakšaiš (60,5 km from the source - the city of Bihać). The amount of Cd in all fish samples was less than 0,1 mg kg^{-1}.

In samples of muscle tissue of trout (*Salmo trutta*) fished in the period of March 2013, the value of Pb was slightly lower and was identified only at the locality of Vrkašić and was 0,1560 mg kg^{-1}. Determined Cu amount in the muscle tissue of fish ranged from 0,4010 mg kg^{-1} to 0,7630 mg kg^{-1}. The minimum value of copper was found at the locality of Orljani, while the largest at the locality of Bakšaiš. The amount of Cd in all samples of fish muscle tissue was less than 0,1 mg kg^{-1}. According to the research by Alić et al.[13] in the trout muscle tissue (*Salmo trutta*) harvested in the River Una, the average amount of Pb was 0,32 mg kg^{-1}, being higher when compared to our results; the amount of Cu in muscle tissue of trout (*Salmo trutta*) was 1,08 mg kg^{-1}, which is lower than the value we found on the locality; the value of Cd in the muscle tissue of trout (*Salmo trutta*) harvested in the River Una was 1,32 mg kg^{-1}, not being in accordance with our research and had a higher value.

CONCLUSION

During the periods of the years 2012 and 2013, the content of heavy metals in water and trout (*Salmo trutta*) of the River Una in observed locations did not exceed the maximum allowed concentrations of heavy metals (Official Gazette BiH37/09)[18].

- The analysis of heavy metals Pb, Cu and Cd in water and fish of the River Una showed that the content was higher downstream

from the town of Bihać compared to the content upstream from the town of Bihać.

- From the analysis, it can be seen that the heavy metal content in 2012 and 2013 was lower than the content of heavy metals determined in the year 2004[13].
- Our results indicate that metal concentrations were very low and that there was no significant pollution of watercourses of the River Una.

It is necessary to closely monitor concentrations of metals during a long period and immediately respond to any changes caused by anthropogenic influence. Only by determining precisely and systematic monitoring of the concentrations of trace metals, we can react on time and preserve this great treasure of ours.

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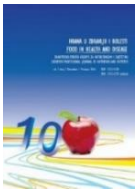

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