TECHNOLOGICA ACTA

ISSN 1840-0426
ISSN 2232-7568

JOURNAL OF SCIENCE PROFESSIONAL FROM CHEMISTRY AND TECHNOLOGY - FACULTY OF TECHNOLOGY TUZLA

Vol. 6
Number 1
June, 2013. year

TECHNOLOGICA ACTA

Journal of Science-professional from Chemistry and Technology Faculty of Technology Tuzla
Vol. 6 Number 1, page 1-74, Tuzla, june 2013. year

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Publisher / Izdavač
Faculty of Technology University in Tuzla

Printing / Štampa
IN SCAN d.o.o. Tuzla

Journal prints two times of year

Technologica Acta is indexed in the following database: CAB Abstracts, COBISS, Index Copernicus Journal Master List, EBSCO

This number of Technologica acta is supported by the Federal Ministry of Education, Science and Culture of Bosnia and Herzegovina

Edition / Tiraž: 200

Editorial Office / Uredništvo
Secretary / Sekretar: Nermina Jahić
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C O N T E N T S

CHANGES IN THE CONTENT OF POLYPHENOLS AND ANTIOXIDANT CAPACITY OF CHOCOLATE LIQUEURS INFLUENCED BY COMPOSITION AND STORAGE

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

With the increasing interest in functional products, liqueurs have become an interesting area of research, trying to confirm the correlation between their chemical composition and beneficial health effects. Chocolate, as a good source of polyphenols, and ethanol, an effective solvent for their extraction, is a good starting point for production of chocolate liqueurs as potentially functional products. The aim of this research was to determine changes in the content of polyphenols and antioxidant capacity of chocolate liqueurs produced from chocolates with cocoa solids content of 30% and 72% in combination with two concentrations of ethanol (20% and 30%) during 3 months of storage. Contents of total phenols, flavonoids, flavan-3-ols and proanthocyanidins were determined spectrophotometrically as well as the antioxidant capacity, using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Initially determined values of the above mentioned parameters were the highest in chocolate liqueur made from chocolate containing 72% of cocoa solids combined with 30% ethanol. Storage did not have marked impact on total phenol content of evaluated liqueurs, although the individual phenolic subgroups exhibited notable changes, both decrease (flavonoids and flavan-3-ols) and increase (proanthocyanidins) in their respective contents.

Key words: chocolate liqueurs, polyphenolic antioxidants, storage

INTRODUCTION

Consumption of various herbal liqueurs for medical purposes has been known since ancient times, while a fact about consumption of chocolate liqueurs dates from the 18th century. Nowadays, liqueurs are very popular beverages usually associated with a luxurious lifestyle and women being as a target group. According to European Council Regulations[1], liqueur is defined as an alcoholic beverage having a minimum sugar content of 100 g/L (as invert), produced by flavouring ethyl alcohol of agricultural origin, or a distillate of agricultural origin or one or more drinks, sweetened, and possibly with the addition of products of agricultural origin such as cream, milk, or other milk products, fruit, wine or flavoured wine. Their alcohol (ethanol) content ranges from 15% to 60% vol., but in practice it usually varies between 17% and 25% vol. Alcohol in liqueurs enables the extraction of different compounds from various flavouring additives, such as spices, nuts and berries, thus contributing to the development of characteristic sweetness, colour, palatability and overall flavour of prepared liqueurs.

One of the most frequently used ingredients for the preparation of liqueurs are cocoa derived products, which consumption contributes to human nutrition through the delivery of lipids, sugars, minerals and antioxidants, principally polyphenols[2]. Cocoa polyphenols have been reported in many studies as bioactive compounds, with antioxidant, antiradical and anticarcinogenic properties[3, 4]. Oligomeric procyanidins isolated from cocoa have been shown to possess biological activities potentially relevant to oxidant defences and immune function[5, 6].

Regarding the mentioned antioxidant value of cocoa derived products reflected by their polyphenolic profile, scientific interest is drawn to conditions at which the extractability and bioavailability of these compounds would be the greatest. Study by Othman et al.[7] showed that ethanolic extracts of cocoa exhibited higher values of antioxidant capacity in comparison to water extracts.

Taking into account that in the production of chocolate liqueurs, cocoa solids are in contact with ethanol, the aim of this research was to determine the influence of the type of chocolate (30% and 72% of cocoa solids) and alcohol content (20% and 30% vol.) on polyphenolic composition and antioxidant capacity of chocolate liqueurs as well as to determine the changes of studied parameters during three months of storage, all in order to produce functional chocolate liqueurs, and thus to provide an additional insight considering the general lack of literature data on this popular beverage.

MATERIALS AND METHODS

Materials

Chemicals

Folin-Ciocalteau, sodium carbonate (anhydrous), ethanol, hydrochloric acid, *n*-butanol, acetone and ammonium ferric sulphate dodecahydrate were of analytical grade and were obtained from Kemika (Zagreb, Croatia). Gallic acid, vanillin and Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) were obtained from Sigma-Aldrich (Steinheim, Germany).

Formaldehyde was purchased from Alkaloid (Skopje, the Republic of Macedonia). Methanol (HPLC grade) was purchased from J.T.Baker (Deventer, Netherlands), and DPPH (1,1-diphenyl-2-picrylhydrazyl) from Fluka (Buchs, Switzerland).

Chocolate cream liqueurs

Liqueurs were formulated in Croatian confectionery company (Zvečevo, Požega, Croatia). Ethanol, in concentrations of 20% and 30%, was mixed with cream liqueur base and homogenized. After the cream liqueur was obtained, 10% of powdered chocolate was added and alcohol content was corrected to final concentrations. Two different chocolates were used for flavouring, dark chocolate with 72% of cocoa solids content, and milk chocolate with 30% of cocoa solids content and 24% of milk solids content. After the final homogenization, 4 different liqueurs were produced and labelled as:

A1- liqueur prepared from dark chocolate and 20% ethanol,

A2- liqueur prepared from dark chocolate and 30% ethanol,

B1- liqueur prepared from milk chocolate and 20% ethanol,

B2- liqueur prepared from milk chocolate and 30% ethanol.

Methods

Determination of total phenol (TPC) and flavonoid (TFC) content

Total phenol content (TPC) was determined spectrophotometrically according to a modified method of Singleton and Rossi[8], using the Folin-Ciocalteau (F-C) reagent. The intensity of blue colouration formation, as a result of the reduction of F-C reagent was measured at 765nm after 2h of the reaction. In order to determine total flavonoid content (TFC), 24h precipitation reaction with formaldehyde in acidic medium was used. Formaldehyde reacts with C-6 or C-8 on 5,7-dihydroxy flavonoids forming condensed products, which were removed by filtration. The remaining phenolic compounds, evaluated as non-flavonoid content (TNFC), were determined according to the previously mentioned procedure for TPC determination. Finally, TFC was calculated as a subtraction of TPC and TNFC. Gallic acid was used as a standard, and the results were expressed as mg of gallic acid equivalents (mg GAE)/L of chocolate liqueur (mean values of two measurements corrected by standard deviations (SD)).

Determination of flavan-3-ol content by vanillin assay

Chocolate liqueurs were analysed for their flavan-3-ol content using a method described by Di Stefano et al.[9]. The intensity of red colouring complex formation after the reaction of 4% (w/v) methanolic solution of vanillin reagent and flavan-3-ol units in acidic medium after 15 min of the reaction (carried out at 4°C in dark) was measured spectrophotometrically at 500 nm. The content of flavan-3-ols was calculated according to the formula: (+)-catechin = 290.8 x ΔA_{500} and the results were expressed as mg of (+)-catechin/L of chocolate liqueur (mean values of two measurements corrected by standard deviation (SD)).

Quantitative determination of proanthocyanidins

Proanthocyanidins were evaluated according to a modified method described by Bate-Smith[10], which is based on the acid hydrolysis of polymeric proanthocyanidins with HCl, resulting in the release of cyanidins of low molecular weight. The mixture of *n*-butanol/HCl solution, diluted sample (in acetone) and 2% (w/v) solution of $NH_4Fe(SO_4)_2$ x $12H_2O$ in 2 mol/L HCl was heated in a water bath at 95°C for 45 min and afterwards cooled and measured for absorbance at 550 nm. The quantity of condensed tannins was determined from a calibration curve of cyanidin chloride and expressed as mg of cyanidin chloride equivalents (CyE)/L of chocolate liqueur (mean values of two measurements corrected by standard deviation (SD)).

Determination of antioxidant capacity: DPPH method

Antioxidant capacity of chocolate liqueurs was determined using DPPH radical scavenging assay described by Brand-Williams et al.[11]. This method is based on the reduction of DPPH (1,1-diphenyl-2-

picrylhydrazyl) radical in the reaction mixture consisting of 0.094 mmol/L methanolic solution of DPPH radical and sample. Discolouration rate of the radical was measured spectrophotometrically at 515 nm against blank (containing methanol instead of the sample). The results were calculated according to Trolox calibration curve and expressed as mmol Trolox/L of chocolate liqueur (mean values of two measurements corrected by standard deviation (SD)).

RESULTS AND DISCUSSION

Total phenol (TPC) and flavonoid (TFC) content of chocolate liqueurs

In the present study, phenolic profile and the antioxidant capacity of four chocolate liqueurs were analyzed on monthly basis during three months of storage. The results obtained within this research follow the recent studies[12, 13] in which it was stated that dark chocolates generally contain more polyphenols than milk chocolates due to their higher cocoa content. As it can be seen in Fig. 1 liqueurs prepared with dark chocolate exhibited significantly higher values of TPC than milk chocolate liqueurs for all 3 measured storage periods.

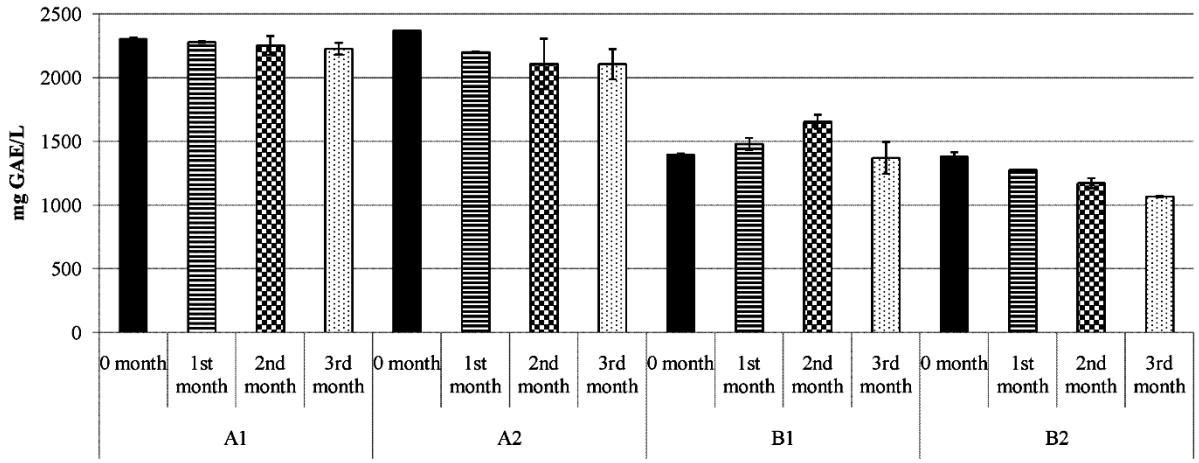

Figure 1. Changes in total phenol content of chocolate liqueurs during three months of storage

The differences regarding the type of chocolate are even more emphasized in TFC (Fig. 2), especially between samples A2 and B2 during all storage time. Again, the obtained results are in favour of higher cocoa content, as expected. Regarding the ethanol content, slightly better extraction of phenolic compounds was obtained in dark chocolate liqueur with 30% ethanol content, at the beginning of storage, while in milk chocolate liqueurs the values were almost the same. Even though it was expected that the prolonged storage would yield to higher TPC values, a general trend was negative, causing a slight decrease of TPC in A1 and A2 samples. In the case of milk chocolate liqueurs, this effect was observable in sample B2, while the lower ethanol concentration (sample B1) exhibited beneficial effect on TPC during first two months of storage, as opposed to all samples. This may indicate that prolonged storage might have induced also a prolonged extraction of phenolic

compounds (20% higher values), but during the third month TPC of B1 also decreased to its initial value. TFC generally followed the trend of TPC with the exception of sample A1 in which the flavonoid losses were most noticeable, resulting in over 50% decrease at the end of storage. Possible explanation for TPC and TFC reduction could be found in reactions of oxidation, polymerization and complex formation of cocoa polyphenols, due to their high reactivity[14]. Higher concentration of ethanol seems to be beneficial for preserving both TPC and TFC in dark chocolate liqueurs, while for milk chocolate liqueurs lower ethanol concentration is essential, exhibiting not only preservation but even better extractability of phenolics, induced by prolonged storage of chocolate liqueurs, but only for 2 months. Differences in TPC and TFC of dark chocolate and milk chocolate liqueurs, regarding the chocolates from which they were prepared, could not be only attributed to the cocoa content of chocolates, but also to the presence of milk solids. Polyphenols represent a highly reactive chemical species that are very likely to react with macromolecules such as proteins and carbohydrates, leading to a different complex formation[15, 16]. As reported by Tong et al.[17] in a study on antioxidant activity of a high molecular weight fraction of whey, polyphenols have high affinity for molecules rich with proline, such as casein. Considering that chocolate liqueurs prepared with milk chocolate contain 24% of milk solids, impact of protein-polyphenol complex reaction cannot be neglected. Besides protein-polyphenol complexes formation, which may have been induced due to the prolonged storage, formation of complexes of polyphenols with sugars is also a possible side reaction that could disable further detection of the polyphenols in liqueur samples. Such outcome could explain a decrease of phenolics during storage.

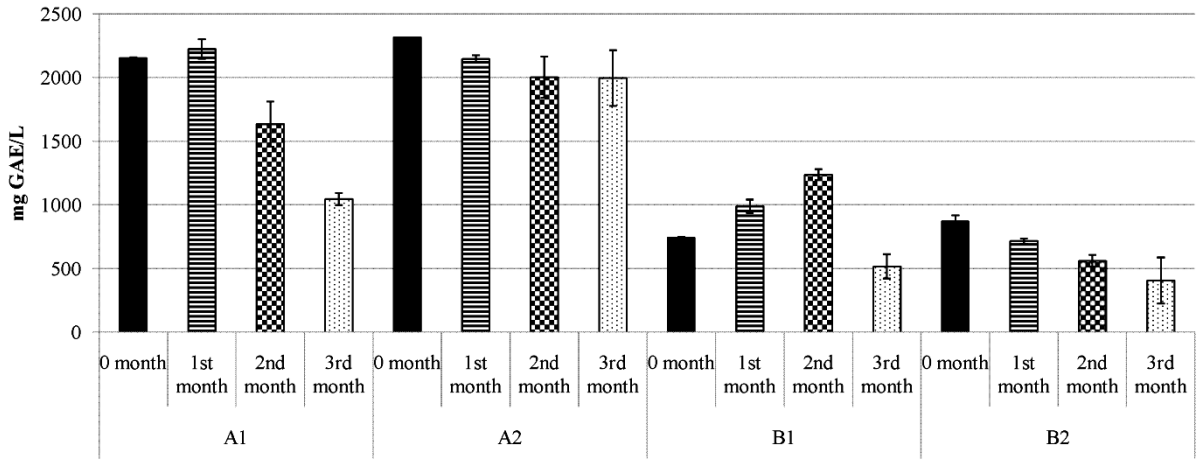

Figure 2. Changes in total flavonoid content of chocolate liqueurs during three months of storage

Proanthocyanidin and flavan-3-ol content in chocolate liqueurs

Comparing flavan-3-ol and proanthocyanidin contents of chocolate liqueurs considered in this study (Fig. 3 and Fig. 4), it can be observed that positive trends during three months of storage are in favour of proanthocyanidins since flavan-3-ol content continuously was

decreasing. The content of both, flavan-3-ols and proanthocyanidins, were more influenced by the cocoa solids content then by different ethanol concentrations. As presented in Fig. 3, content of flavan-3-ols in dark chocolate liqueurs was initially markedly higher in comparison to the values obtained for milk chocolate liqueurs, which also remained during storage. Analysis of sample A1 resulted in linear decrease of flavan-3-ols, while for sample A2 a mild increase after the first month was observed, but also finally resulted in flavan-3-ol decrease. Milk chocolate liqueurs exhibited negative trend of flavan-3-ol contents during storage as well. The effect of different ethanol concentration was most noticeable regarding the rate of flavan-3-ol losses. Higher ethanol concentration in dark chocolate liqueurs enabled better preservation of these compounds, while in milk chocolate liqueurs stagnation in flavan-3-ol degradation was obtained with lower ethanol concentration.

As can be seen in Fig. 4, determined content of proanthocyanidins in dark chocolate liqueurs at the beginning of storage was markedly higher, exhibiting values of 811.27 mg CyE/L and 906.76 mg CyE/L for samples A1 and A2 respectively. During storage, a mild increase in proanthocyanidin content was observed for both samples, which yielded to 1214.33 CyE/L for sample A1 and to 1051.40 CyE/L for sample A2. Although the initial proanthocyanidin content in A1 was lower than in A2, lower concentration of ethanol helped obtaining higher final values. Initial values of proanthocyanidins in milk chocolate liqueurs were very similar with the lowest value of 84.36 CyE/L found in sample B1 (Fig. 4).

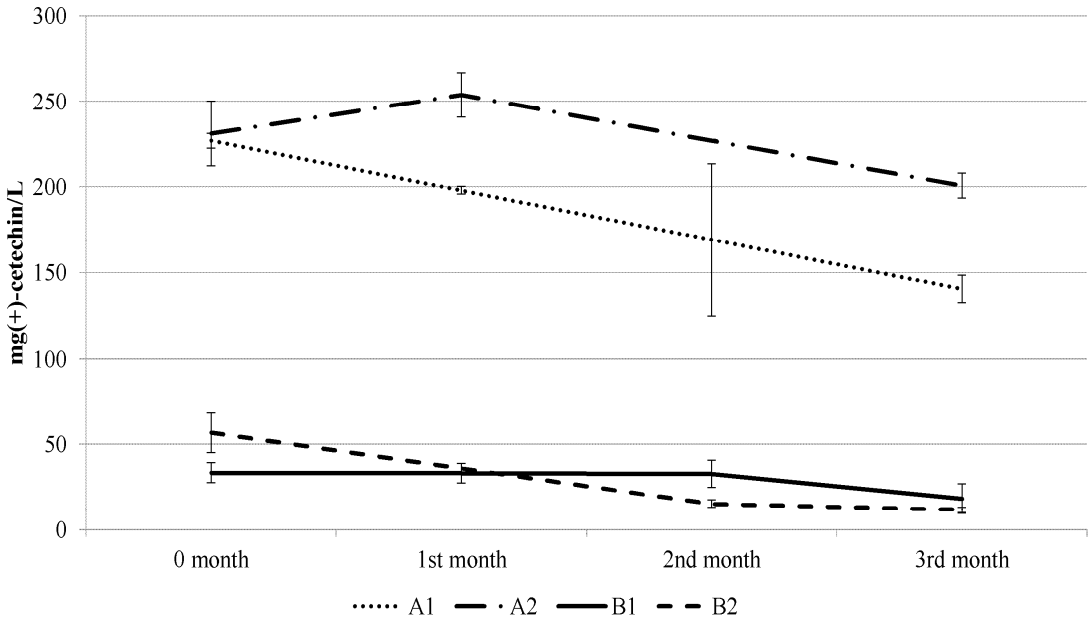

Figure 3. Changes in flavan-3-ol content of chocolate liqueurs during three month of storage

Both samples followed linear increasing trend during storage, exhibiting almost the same values, with the exception of a slight decrease during the 3rd month in sample B1. The obtained results considering proanthocyanidins could be explained with

findings of Belščak et al.[18] who stated that the extended extraction of polyphenols from cocoa derived products usually yields in proanthocyanidins of higher molecular mass and more conjugated structure, built from polymerized flavan-3-ol chains.

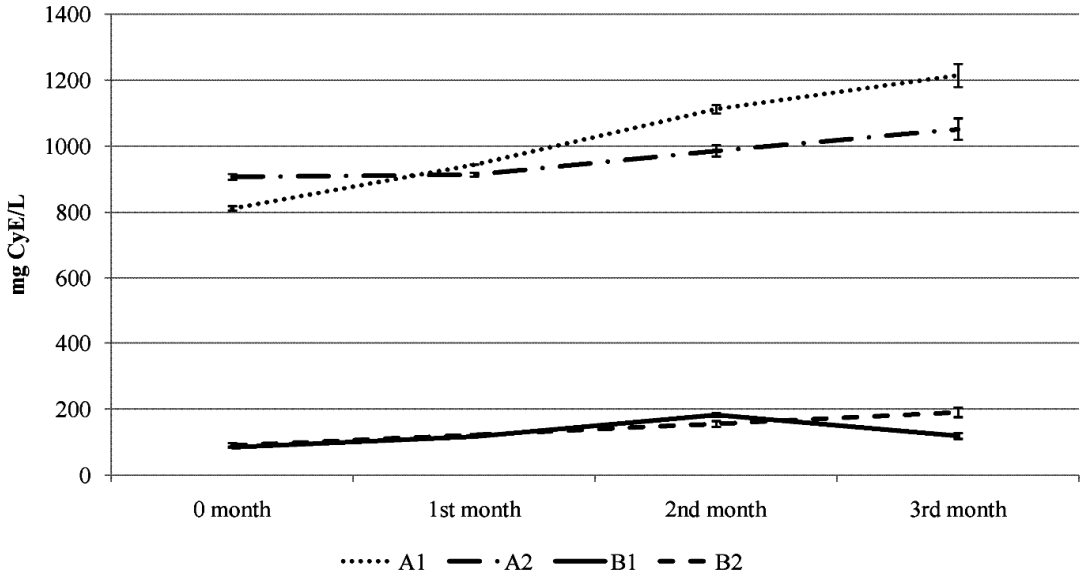

Figure 4. Changes in proanthocyanidin content of chocolate liqueurs during three month of storage

Antioxidant capacity of chocolate liqueurs

As previously reported in numerous studies[18, 19, 20], cocoa bean and cocoa derived products represent a noteworthy source of polyphenols. Among them, flavonoids possess a tremendous antioxidant capacity due to their chemical structure, capable of releasing the hydrogen atom and becoming a radical without losing its stability[21, 22, 23]. As shown in Table 1, values attained by DPPH assay were generally higher in dark chocolate liqueurs, among which sample A2 exhibited the highest antioxidant potential at the beginning of storage, followed by slight fluctuations to a final value, comparable to the initial one. Antioxidant capacity of sample A1, although initially lower than of A2, exhibited an increase in DPPH values, yielding up to 88% of A2 final value. Regarding milk chocolate liqueurs, positive influence of prolonged storage in DPPH values was observed as well. The rate of antioxidant capacity increase showed no marked deviations between samples B1 and B2, resulting with final values similar to the initial value of sample A1. The above mentioned imposes ethanol of higher concentration to be beneficial for maintaining a certain level of antioxidant capacity of dark chocolate liqueurs during storage, while for milk chocolate liqueurs the impact of two ethanol concentrations was not as notable. It is well established that cocoa procyanidins exhibit higher antioxidant potential than most of the monomeric polyphenolic compounds found in cocoa[24]. Bravo[25] has reported that non-extractable polyphenols, such as polymerized proanthocyanidins and tannins are even 15 to 30-fold more effective in free radical quenching, which can be applied to this study, implying that extraction of polyphenolic compounds with higher molecule mass and stronger

antioxidant capacity was enabled due to the prolonged storage.

Table 1. Changes in antioxidant capacity of chocolate liqueurs during three months of storage

SAMPLE	Storage time	DPPH (mmol Trolox/L ± SD)
A1	0 month	3.45 ± 0.30
	1st month	5.17 ± 0.13
	2nd month	6.83 ± 0.30
	3rd month	8.62 ± 0.05
A2	0 month	9.90 ± 0.12
	1st month	10.55 ± 0.09
	2nd month	9.19 ± 0.04
	3rd month	9.79 ± 0.77
B1	0 month	0.80 ± 0.05
	1st month	1.58 ± 0.07
	2nd month	2.70 ± 0.02
	3rd month	3.15 ± 0.25
B2	0 month	1.16 ± 0.06
	1st month	2.05 ± 0.02
	2nd month	2.93 ± 0.42
	3rd month	3.83 ± 0.20

CONCLUSION

Chocolate liqueur prepared with chocolate containing 72% of cocoa solids in combination with 30% ethanol was proven to have the highest content of all measured parameters which was accompanied with the best preservation of the latter during three months of storage. Similar observations can be made for dark chocolate liqueur containing 20% of ethanol as well. Considerable antioxidant potential of all chocolate liqueurs indicates the need for further development and research in order to produce functional products.

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FACTORS AFFECTING THE CONCENTRATION OF UREA IN MILK

REVIEW ARTICLE

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ABSTRACT

Today, in addition to all the problems that follow the dairy sector, the producers will have to pay particular attention to environmental protection. All louder are the different organizations that warn of the cattle farm as one of the environmental issues.

On the other hand, incorrect use and non-balanced protein diet indirectly leads to these problems. Sources of crude protein, which is used for feeding of cows, at the present time, are quite expensive and directly affect the price increase of milk production. In addition to food as the most important, other factors affect the concentration of urea in milk as well and they should not be neglected. In this paper we present some of these factors and present the works of various authors on the subject. The first and major factor which directly affects the concentration of urea is production of milk. Then come other non-nutritional factors such as seasonal conditions, the number of lactation and lactation stage.

In recent years we are witnessing the unfavorable weather conditions that have a negative affect on milk production and therefore should emphasize the importance of heat stress, as well as factors associated with the concentration of urea in milk.

Key words: milk, heat stress, urea

Literature review

Milk is a specific product of mammary gland. It is a complex product affected by so many factors. Its composition is affected by a lot of parameters such as the breed, the stage of lactation, season, mode of nutrition, housing conditions and much more. Relationships between particular milk components are mostly unchangeable and can serve as parameters if some problems occur in production. Disrupted relations are the true sign of trouble whether in feeding or management of the farm.

Urea, by itself, is a normal component of milk as a part of non-protein nitrogen. Marenjak et al. (2004) concluded that the unbalanced diets of cows, especially with an excess of proteins, is an economic loss in production. Moreover, it is inefficient for animals as well as unfit for the environment.

The same authors note that the concentration of urea in the blood and milk increases with the energy deficit and insufficient supply of easily digestible carbohydrates, excessive protein supply and absence of energy combined with an excess amount of protein in the diet.

Jonker et. al. (2002) state that the milk urea can be used as a tool to improve herd nutrition and to monitor the nutritional status of lactating dairy cows. Unuk (2003) states that the proper evaluation of forage meals is defined by the content of protein, fat and urea in milk. He especially emphasizes that the urea concentration in milk is always observed together with a protein content of

milk and the recommended values represent the optimum supply of animal with raw protein and energy.

When the protein content of the milk is in the range from 3.2% to 3.8%, the normal values of urea concentration are within the range of 15 and 30 mg / dl. (Pintić et al., 2007). Different authors present different information about the content of urea concentration in milk. Jonker et al. (1999) refer to, as normal values of urea in milk the concentration from 10-16 mg / dl; Kohn et al. (2004) consider the normal values of urea concentration from 7 to 19 mg / dL. Babnik et al. (2004) report that it is the value of 15 to 30 mg / dl. For normal values of urea one group of scientists say it is from 0-15 mg / dl (Moore and Varga, 1996). The concentration of urea in milk is correlated with the level of crude protein intake from diet, the percentage of degradable and non-degradable rosy proteins and the relationship between protein and energy (Röseler et al., 1993, Baker et al., 1995), and negative correlation of the intake of non-fibrous carbohydrates in the diet of cows (Godden et al., 2001).

Milk production

Early studies related to the correlation between milk production and concentration of urea in milk present different views. Most reports have found a positive correlation between milk production and urea concentration (Kaufmann, 2001, Macleod et al., 1984; Oltner et al., 1985, Carlsson et al., 1995; Godden et al., 2001; Arunvipas et al., 2003; Hojman et al. , 2004), while other authors in their works had not found significant correlation (Gustaffson and Palmquist, 1993; Gustaffson and Carlson, 1993, Baker et al., 1995; Eicher et al., 1999a). Trevaskis and Fulkerson (1999), also in their work point out negative correlation between urea content and milk production.

According to researches the urea concentration in milk and daily milk production level are positively linked in herds with high average of milk production (> 10 000 kg of milk per cow in lactation), unlike the herds with lower average of milk production per cow (<7000 kg milk) for which no significant correlation was found (Rajala-Schultz and Saville, 2003). It is also found, in herds with high average lactation of milk production, significantly higher average of urea concentration in milk, which the authors explained by the level of crude proteins in meals of high dairy cows.

Increasing of milk production for 2 000 kg in lactation is associated with the increase of the level of urea in milk by 2.6 mg / dl (Jonker et al., 1999), and 0.33 mg / dl (Arunvipas et al., 2003).

Positive correlation between daily milk production and concentration of urea in milk could be the result of increased production that results in higher nutritional needs for crude protein. Addition of proteins in meals increases milk production by providing more amino acids necessary for the synthesis of milk proteins, increasing amounts of easily digestible carbohydrates in the diet of cows. Macleod et al. (1984) suggest that by increasing the level of crude protein in the diet increases the intake of dry forage, which indirectly results in increasing the energy with the meal intake.

Sackett et al., (1991) report that the majority of studies conducted on individual animals in experimental conditions using tests to measure the urea concentration in milk. The

link between urea and feed ingredients should be determined in field conditions in which a standard test procedure is performed.

Season

Season has an impact on the concentration of urea in milk. According to research during the summer period, total nitrogen content (total nitrogen, TN) and true protein (mainly casein) in milk decrease, whereas the content of non-protein nitrogen (NPN), which includes urea, increases (Carlsson et al., 1995, Ferguson et al., 1997). According to other authors a significant interaction between season and milk production in cows kept on pasture was determind (Rajala-Schultz and Saville, 2003). Cows of lower milk production (<7258 kg of milk per lactation) milk urea concentration was significantly higher during the summer. It is well known that fresh pasture contains of digestible protein, and a high ratio between energy and proteins (Soriano et al., 2001). High producing herd of cows (> 10 433 kg of milk per cow per lactation) had low urea concentration during the summer months due to reduced dry fodder intake or feed intake proteins because of the heat (Rajala-Schultz and Saville, 2003). Based on the research of Hojman et al. (2004) on cows kept all year round in the barn and fed with a complete total mixed ratio (TMR) meal without green (mowed) grass, the minimum value of urea were found just over the summer, and the authors speculate that the effect of season on the urea concentration is direct. Hojman et al. (2004) suggest that the concentration of urea in milk is higher in spring and the highest at the beginning of summer in June (18.1 mg / dL). Similar results were obtained by Abdouli et al. (2008). Konjačić et al. (2010) showed that the season has a significant impact on the concentration of urea in milk, which concentration in summer and autumn was higher than in winter and spring. Jilek et al. (2005) got in their works higher concentration of urea in milk during the winter.

Number of Lactation

Researching the individual milk samples, some authors have found a significant impact of the number of lactation on the concentration of urea in milk and blood of cows, and that with increasing the number of lactation, the concentration of urea in milk increased significantly (Oltner et al., 1985, Carroll et al., 1988, Canfield et al., 1990, Barton et al., 1996; Godden et al., 2001; Arunvipas et al., 2003; Hojman et al., 2004).
Cows in first lactation are still in the stage of growth and development, and therefore, more efficiently exploit the amino acids from the diet. The result could be reduced deamination and urea synthesis in the liver, resulting in lower milk urea concentration in cows in first lactation (Oltner et al., 1985).
According to Carlson et al. (1995) cows of second or higher number of lactation have a higher concentration of urea in milk than the first calving ones but only when they are placed in the barn, in comparison to cows kept on pasture. Johnson and Young (2003) state that Holstein cows have the highest content of urea in milk in the first lactation,whereas in Jersey cows have not be found a significant difference between lactations.
On the other hand, other studies indicated a very small, statistically insignificant

differences, due to the increased number of lactation (Canfield et al., 1990.; Gooden et al. (2001a)

Stage of lactation

Urea concentration in milk varies according to the stage of lactation (Oltner and Witkorsson, 1983; Oltner et al., 1985, Carlson et al., 1995, Moore and Varga, 1996; Godden et al., 2001-a; Rajala-Schultz and Saville, 2003). The concentration of urea in milk is lowest during early lactation (first 30 days), while in cows kept on pasture the lowest concentration of urea in milk was found between the 40^{th} and 60^{th} days of lactation (Trevaskis and Fulkerson, 1999).

Lower urea concentration in milk can be related with reduced dry matter intake in the period immediately after giving birth, and a sharp rise in milk production, especially in the high productive dairy cows (Carlsson et al., 1995). The same authors reported that late lactation, with reduced milk production is reduced in need for protein, normally with sufficient degradable protein and carbohydrates in the diet. Urea concentration in milk generally follows the curve of lactation (Jonker et al., 1999, Johnson and Young, 2003), and urea in milk reached a peak just in time of the highest lactation of milk production (Rajala-Schultz and Saville, 2003) .

According to Carlsson et al. (1995) and Arunvipasu et al. (2003) the concentration of urea in milk reaches a peak between 3 and 6 months of lactation. Due to the significant interaction between number and stage of lactation (Carlsson et al., 1995; Godden et al., 2001-b), a drop of urea concentration in milk during late lactation was significantly higher in cow in second or higher number of lactation than cows in first lactation.

Gantner et al. (2006) found that the adequate supply of digestible protein and energy was detected in 13.79% of cows in the early stage of lactation, 16.36% in the middle stages of lactation, and 24.75% in the last stages of lactation. In the initial stage of lactation, i.e. in the first 60 days it is evident the insufficient energy supply, while the surplus is characteristic when duration of lactation is longer than 120 days.

Emanuelson et al. (1993) have come to the conclusion that the highest concentration of urea in milk is between 60 and 90 days of lactation. In studies of Koljančića et al (2010) the proportion of urea was the lowest in the first calving cows (27.34 mg / dL) and significantly increased in the cows of higher level of lactation.

Mid-lactation (100-200 days) recorded the largest, and in late lactation (> 200 days) was the lowest average of urea content in milk. Schepers and Meijer (1998) did not find any significant correlation between stage of lactation and urea concentration in milk in identical conditions of feeding of the experimental group of cows in the study. Wood et al. (2003) referred for the cows of the first three lactation levels that the concentration of urea in milk is inverse to the movement of lactation in milk production, with the highest values of urea at the end of lactation.

A similar positive relationship between the stage of lactation and concentration of urea in milk was cited by other authors (Ng-Kwai-Hang et al., 1985; DePeters and Cant, 1992; Broderick and Clayton, 1997; Giger et al.,1997; Hojman et al., 2004). In late lactation, due to reduced milk production, cow's protein needs are falling, and thus the

concentration of urea in milk, of course, assuming sufficient amounts of rumen degradable protein and an optimal ratio of protein and fermentable carbohydrates in the diet. This points to the fact that non-nutritional factors are of minor importance for the explanation of the relationship between stage of lactation and milk urea concentration (Schepers and Meijer, 1998).

In the case of feeding the cows in late lactation, with raw diet too rich in protein or low in energy, milk urea concentration will grow in parallel with the decline of milk production.

In contrast to his previous works, Faust et al. (1997), and Schepers and Meijer (1998), found no variation in concentration of urea in milk in relation to the stage of lactation. Cows in early lactation in ruminal flora have not yet been adjusted to move to the high protein meal after calving. Consequentially the mismatch of energy and protein may lead to increase of urea concentration in milk in the first months of lactation (Jorritsma et al. 2003).

Influence of thermal stress on the urea concentration

Heat stress is nowadays a serious problem, because the global warming becomes more and more important. This is a major health and economic problem, especially in the summer months because of reduced milk production at the farms which leads to reduction of the percentage of milk fat, protein and non-fat solids in milk causing the great economic loss (Cincović and Belie, 2009). Urea in the blood comes from the rumen or from muscle tissue. It is produced in the liver from ammonia which is produced during degradation of proteins in the fore stomach, or due to protein catabolism and utilization of amino acids in the process of gluconeogenesis, which is characteristic of metabolic realignment during heat stress (Ronchi et al., 1999, Jenkins and McGuire, 2006; Cincović et al., 2010). Studies of Spiers et al., 2004, showed that cows in the second and third level of lactation significantly reduce milk production during heat stress, because in this period, milk production depends on food intake.

Belić et al., 2011, state that the degradation of glucose for energy needs provides less energy than the digestion of fat, which reduces the thermal load on the body. The same authors suggest that during heat stress cows used more glucose for energy purposes rather than for the production of milk, while exploiting the significant fat for milk production. Because of protein catabolism and excess of ammonia from the rumen, urea production is increased in the liver of cows during the impact of heat stress (Ronchi et al., 1999; Cincović et al., 2010). Urea is filtered easily through cell membranes and has numerous effects on milk production. The assumption is that a high concentration of urea and its passage through the udder has a negative impact on the production (Belic et al, 2011). Belić et al., 2011, found that a concentration of urea is significantly higher in the thermal stress. Since urea readily passes through the cell membrane, easily gets into the milk, its excretion decreased during the action of heat stress.

CONCLUSION

The great importance for control of milk urea concentration was obviously. This could be one of the tools to control the nutritional status of the cows and its improvement. In this way, we can

significantly reduce the cost of milk production. Many authors have stated a positive relationship between increased production and concentration of urea in milk. With the influence of season, it is evident that in the summer months there is lower concentration of urea, besides the satisfaction of other factors and is subject to significant variations. With regard to the tage of lactation there are variations observed in urea concentration. When increasing number of lactation urea concentration increases. The amount of urea in the diet affects the increase of concentration of nitrogen, and thus the negative effect on the environment. The assumption is that high concentrations of urea and its passage through the udder have a negative impact on the production in dairy cows.

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EFFICIENT ENERGY CONSUMPTION REDUCES NEGATIVE IMAPCTS ON THE ENVIRONMENT

REVIEW ARTICLE

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ABSTRACT

Analysing the current air quality, it has been concluded that heating of individual residential buildings affects air pollution in winter. Experiences of European countries in the implementation of energy efficiency measures in buildings and their contribution to the reduction of energy consumption and negative impacts on the environment were taken into account.
The research was conducted on a sample of 50 typical individual residential buildings. As a part of the research, a detailed energy audit of all buildings was conducted; consumption of heating energy – generating products for two heating seasons and indoor and outdoor temperatures for all buildings were monitored.
Rehabilitation measures were developed and proposed based on collected data after the first heating season and energy balance.
The analysis of collected data on the quantities of spent fuel and heated area in all 50 buildings has shown that the buildings on average consume 338 kWh/m^2 of heating energy, while similar buildings in Europe consume 200-300 kWh/m^2.
After the rehabilitation of 50 buildings, average consumption of energy – generating products and CO_2 emissions were reduced by 42% and 46% respectively, but also other gases emissions caused by coal combustion were reduced as well.

Key words: energy efficiency, thermal insulation, insulation materials, energy balance, Infrared thermography.

INTRODUCTION

Energy management system is a set of processes which allows us use of data and information to maintain and improve energy efficiency, increase operational efficiency, reduce energy intensity and negative impact on the environment.
In this paper based on the research conducted in the housing sector, we present that there are significant reserves and opportunities to improve energy efficiency and reduce negative impacts on the environment both in housing and other industrial and service sectors.

High concentrations of various air pollutants, substantially increased during the heating season, indicate that both individual and collective furnaces, in addition to using a lot of energy, are one of the major pollutants which affect health.
The results of the research on adverse health effects of air pollution have shown that excessive air pollution is correlated with cardiovascular and respiratory mortality[1].
The study on air quality in Tuzla[2] has shown that the regular monitoring of the air confirms the existence of hazardous substances which often exceeds the limit

values. Concentrations of air pollutants exceed the limit value, laid down by law, especially in the winter period. For example, at the air measurement station Skver in Tuzla, in January 2011, a mean value of SO_2 daily concentration was 189,4 $\mu g/m^3$, and its maximum value was 520,8 $\mu g/m^3$, but an annual mean limit value is 90 $\mu g/m^3$.

According to the EU Directive 2002/91/EC[3] on the energy performance of buildings member states are required to implement it.

Annual heating energy consumption is approximately 250 kWh/m² in the old buildings in EU; standard insulated buildings spend less than 100 kWh/m²; modern low energy houses spend about 40 kWh/m²; passive houses 15 kWh/m² and less[4]. These differences in energy requirements indicate the possibility of efficient energy consumption in the building industry.

According to the usual construction procedures buildings should be thermally insulated because basic building materials, which provide bearing and strength capacity, conduct more heat than acceptable.

The thermal insulation should reduce energy consumption; protect the environment, and bearing capacity from external weather conditions.

ECOLOGICAL AND ENERGY EFFICIENT CONSTRUICTION

Knowledge of the thermal properties of building materials is one of the prerequisites for the design of energy-efficient buildings. Heat loss through building materials depend on the composition of material and the thermal conductivity coefficient. Better thermal insulation is achieved by installing materials with low thermal conductivity and high thermal resistance. Thermal resistance of the material is proportional to the thickness of the material.

Coefficient of heat conductivity, U, is heat which construction element loses in 1 second per m^2, at temperature difference of 1 K, expressed as W/m^2K. Coefficient U is very important property of construction and play important role in analyses of total heat loses (kWh/m^2), as well as in energy consumption for heating. As this coefficient higher, heat protection is better.

The designing stage of constructing new buildings and residential buildings should predict all necessary issues in order to construct a quality and optimal energy-efficient building. Therefore, it is necessary to:

- to analise a site, position and form of a bulidng,
- to apply the high level of thermal insulation of the whole external enclosure and avoid thermal bridges,
- to use the sun heat gains and protect from over exposure to sunlight,
- to use energy efficient system of heating, cooling and ventilation in combination with renewable energy sources,
- to use construction materials which are not harmful to the environemnt (energy and eclogical effective materials),
- to properly design chimnyes[5].

Qualitative installation of insulation materials around the entire outer layer of a building without any interruption is of great importance in order to prevent creation of thermal bridges.

In addition to installing thermal insulation in energy efficient buildings, it is necessary

to install insulation material which prevents air leakage, found in most of thermal insulation materials, or provide air tightness by a construction itself.

The exsisting buildings represent a huge potential for energy and ecological savings since a great number of bulidings have inadequate thermal insulation. More than 85% of buildings in Bosnia and Herzegovina have inadequate thermal insulation[6] with the average heating energy consumption of more than 200 kWh/m^2.

Heating energy consumption in buildings

Energy Sector Study in Bosnia and Herzegovina[7] suggested that nonrational energy consumption for heating, cooling, ventilation, etc., is characterisic for almost all types of residential and public buildings.

The building secotor including households and service sector is the largest individual consumer of energy on the entire teritory of Bosnia and Herzegovina (Figure 1). Rising standard of living is likely to further increase energy consumption for heating, cooling and also for electricity used to operate increasing number of appliances[8].

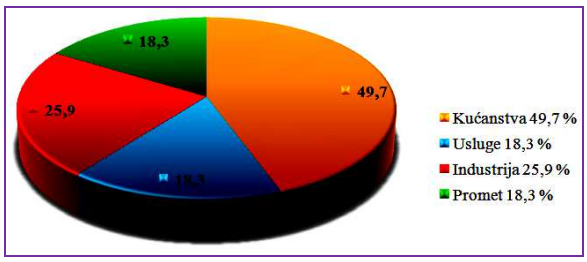

Figure 1. Energy consumption in sectors in 2012 for B&H[8]

The energy in residental buildings of the European countries is used at about 57% for space heating, 25% for hot water and 11% for electrical appliances and lighting.

The experiences of European countries have shown that buildings offer the greatest potential for energy savings. Increased level of thermal insulation of buildings using energy efficient materials can significantly reduce heat loss and total energy consumption.

BUILDINGS AND CLIMATE CHANGE

Buildings are responsible for 40% of energy consumption in the world, and for more than 50% of greenhouse gas emissions form buildings and building related transportation.[9] Great amounts of greenhouse gas emissions from buildings contribute to climatic changes, which are increasingly greater and pose a threat to mankind. Therefore, the challenge of the 21st century designing is an improvement of energy efficiency which further improves the environmental quality and contributes to the global struggle against climatic changes.[10]

If we consider the emission of hazardous substances into air in the building sector since 1990, we see an increasing trend of CO_2 emissions caused by fossil fules from production processes and other sources (Figure 2).

Energy efficiency includes a wide range of activities with the overall objective of reducing consumption of all types of energy in the buildng resulting in CO_2 emission reduction.

Problems caused by global greenhouse gas emissions (global warming and climate changes) require that the trend in CO_2 emissions is taken into account.

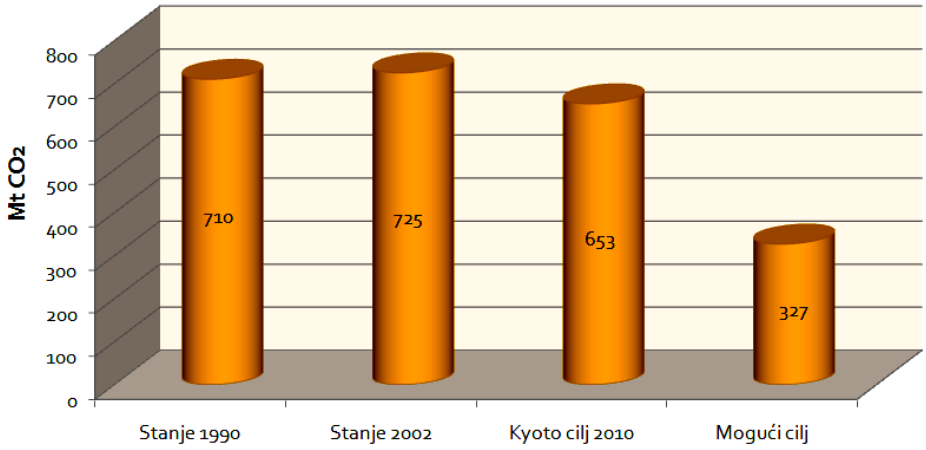

Figure 2. Emission of CO_2 in the building sector of EU[5]

Technical regulations on heating energy savings and thermal protection in buildings

The first regulation on thermal protection of buildings, Regulation on Technical Measures and Conditions for Thermal Protection of Buildings, was adopted in 1970.[11] The Regulation stipulates the maximum heat transfer values U (W/m^2K) of individual building components with respect to the climate zone in which the building is located.

Different legislations in different countries stipulate the conditions on thermal protection according to the standard of individual markets.

Table 1 shows the heat transfer coefficient in the building sector for some countries and Bosnia and Herzegovina.

Table 1. Allowed heat transfer coefficient, U (W/m^2K), for wall, floor and ceiling

Country	External wall	Floor	Ceililng
Swiss	0.4	0.4	0.4
Sweden	0.3	0.3	0.3
Germany	0.38	0.3	0.38
Denmark	0.27	0.3	0.2 – 0.3
England	0.45	0.45	0.25 – 0.45
USA	0.47	0.58	0.22
Bosnia and Herzegovina	**0.60**	**0.50**	**0.40**

The main objecitve of energy efficency in buildings is to establish mechanisms which will lead to the permanent reduction of energy with projecting, construciton and using of new residential buildings, as well as in reconstruction of exisiting.

RESEARCH RESULTS

In this study, 50 typical residential buildings constructed of various building materials with individual heating systems consuming large amounts of energy-generating products (coal, lignite and wood) were selected.

Infrared thermography

All 50 buildings were photographed by infrared camera, and thermographic images showed significant heat loss. Particularly

critical places of heat loss are horizontal and vertical armoured concrete beams and concrete lintels above doors and windows.

Figure 3 shows critical places of heat loss through the outer enclosure, typical for all buildings, with the high heat transfer coefficient, leading to high energy losses.

In addition to losses through the outer enclosure, heat losses occurred through chimneys and pipes, because of old boilers and insufficient thermal insulation. Boilers are not maintained according to the technical guidelines and are generally oversized, which is particularly evident in the period after rehabilitation. Pipelines are usually not insulated as well.

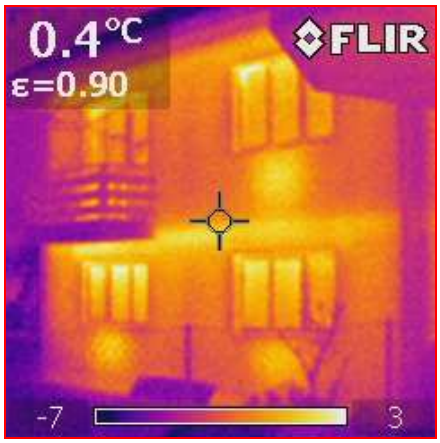

Figure 3. Typical heat losses through the outer enclosure of buildings recorded by IC camera

Frequently, the pipelines that run through a basement are not insulated, so that the basement unnecessarily achieves temperature of heated space.

Therefore, the technical failures cause great heat losses. In buildings with the chimneys outside the building, significant heat losses were observed (Figure 4).

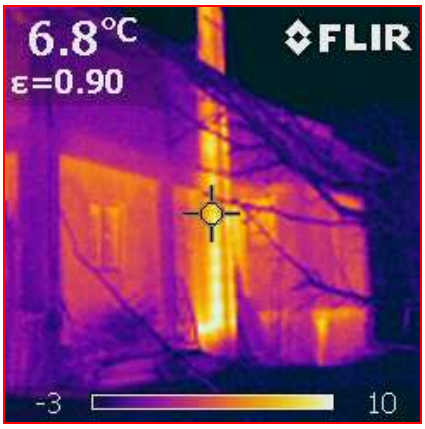

Figure 4. Heat losses through chimney

Energy–generating product consumption

Energy – generating product consumption was measured in all buildings before and after the thermal rehabilitation of buildings. Data analysis showed that the buildings prior to the rehabilitation spent 338 kWh/m^2 on average, while after the rehabilitation 196 kWh/m^2 resulting in decreased energy consumption of approximately 42%. The comparison of the results of thermal energy consumption in MJ/year for fifty buildings before and after rehabilitation is shown in Figure 5. The consumption of energy-generating products has been significantly reduced per m^2 of heated space after the rehabilitation. However, the comparison of the results with the European standards suggested that energy consumption remains high. Therefore, further analysis was done and possible causes were identified:

- frequent heating before the rehabilitation,
- too high indoor temperatures (an average temperature was approximately 25 °C),
- heat losses through doors and windows,
- inadequate ventilation and aeration,
- too big furnaces, and
- poor combustion, improper heating as well as heat losses through pipelines.

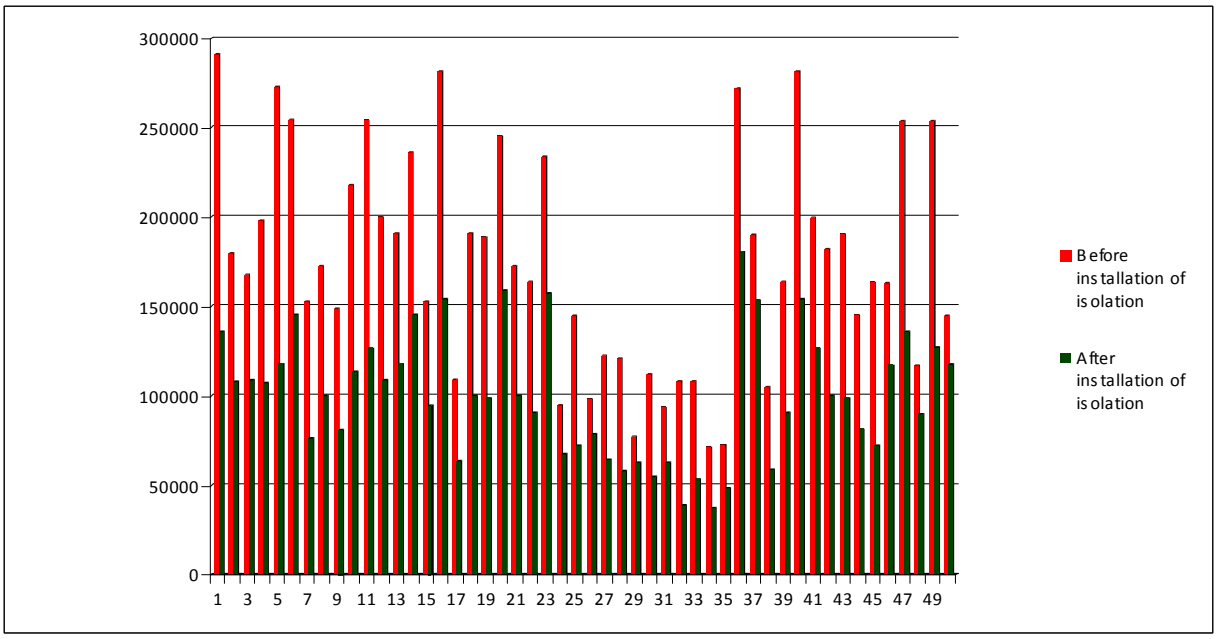

Figure 5. Results of measuring of heat energy consumption in MJ per year for fifty buildings before and after installing the insulation

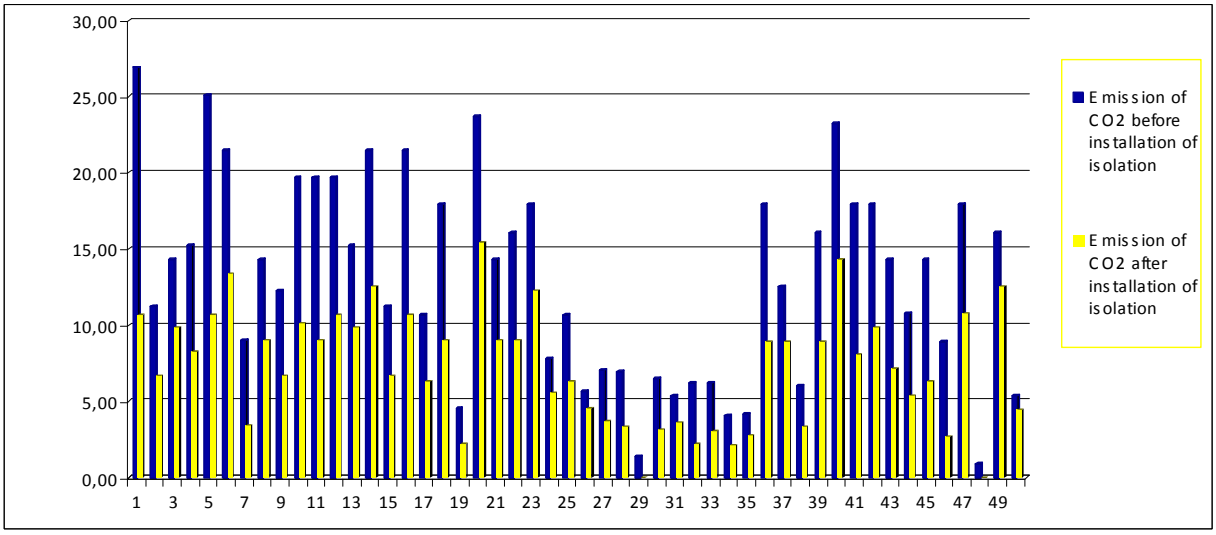

Figure 6. CO_2 emissions before and after installing the insulation for fifty buildings

CO_2 Emissions

According to the Guidelines of Federal Ministry of Environment and Tourism of Bosnian and Herzegovina and based on a type and amount of consumed energy - generating products, CO_2 emissions were estimated for all buildings before and after rehabilitation (Figure 6).

The result analysis showed that the buildings emitted 13 and 7 tonnes of CO_2 before and after rehabilitation respectively, indicating that the emission was reduced by 46%.

An average emission of CO_2 per m^2 of heating space was 87 kg before the rehabilitation. However, it was reduced to 48 kg after rehabilitation.

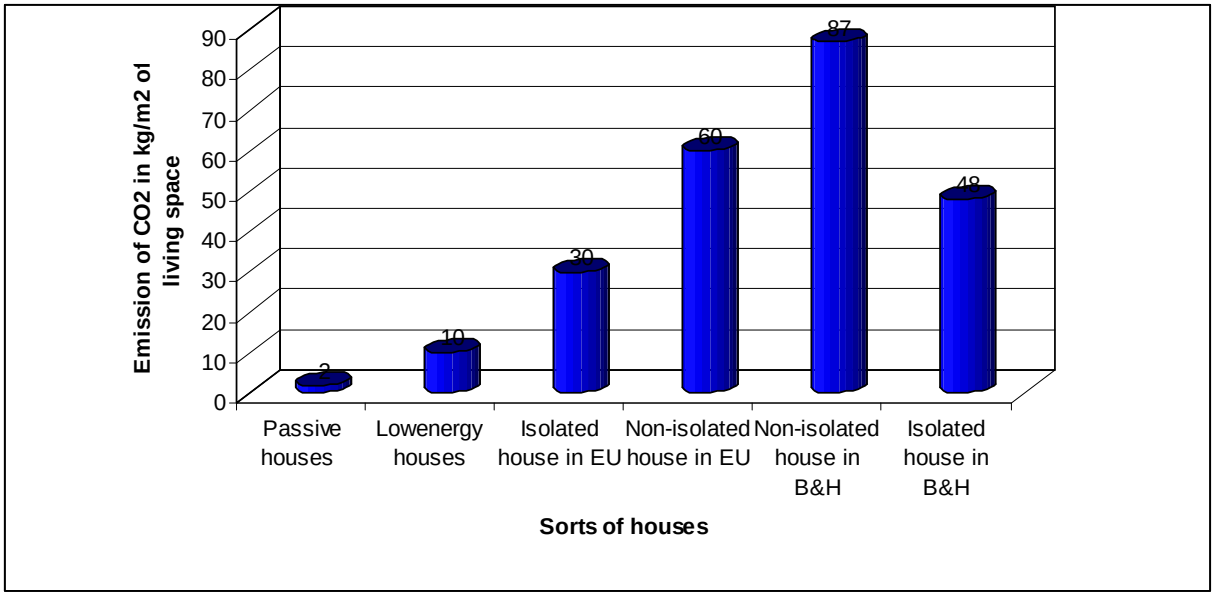

Figure 7. Emission of CO_2/m^2 of living space

Comparing our results with the EU results, it can be seen that the CO_2 emission per m^2 of heating space is still too high in Bosnia and Herzegovina (Figure 7).

In Bosnia and Herzegovina, a passive house annually emits 2 kg/m^2 of CO_2, a house without thermal insulation 60 kg/m^2, a house built in the 70s and 80s approximately 30 kg/m^2, while a non-insulated house emits 87 kg/m^2, and insulated approximately 48 kg/m^2 of CO_2.

The high CO_2 emissions are caused by coal used for heating of buildings which is not used in the EU countries.

CONCLUSION

Based on the research conducted in 50 residential buildings it was found that non insulated buildings consume 338 KWh/m^2 of heating energy while similar buildings in Europe consume 200 – 300 kWh/m^2.

The technical failures on buildings, such as:

- bad construction at vertical and horizontal armoured concrete beams, as well as concrete lintels above doors and windows,
- improper ventilation and aeration,
- poor combustion in furnaces, improper heating, and heat losses through the pipes and furnaces.

are causes of high energy consumption.

After the rehabilitation of 50 buildings, average consumption of energy – generating products and CO_2 emissions were reduced by 42% and 46% respectively, which is significant from economic and ecological point of view.

This study has shown that heating energy consumption can be reduced applying simple energy efficient measures, and thereby the negative impact on the environment.

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EFFECTS OF ACTIVATED CARBON TYPES ON NOM REMOVAL EFFECT FROM NATURAL WATERS

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

Natural waters of eastern Croatia, groundwater and the river Drava water, contain an increased concentration of natural organic matter (NOM) as a result of complex animal and vegetable dissolving processes in the environment. Since the increased NOM concentration in drinking water causes appearance of yellowish colour and formation of trihalomethanes (THM) during water chlorination, the main task of water-treatment process, is to remove NOM from water which often implies water filtration through activated carbon filters.
In order to examine the efficiency of NOM removal from natural waters, four granulated activated carbons were tested and the main factors affecting adsorption efficiency, e.g., carbon dosage, pH and contact time were studied.

Key words: NOM removal, activated carbon, adsorption

INTRODUCTION

Natural organic matter (NOM) is a mixture of organic compounds, such as proteins, amino acids, carbohydrates, humic and fulvic acids, derived primarily from plant and/or microbial residues, present in all surface and ground waters on earth with concentration ranging from 0,5 up to 10 mg/L.

Besides the natural sources, human inputs, such as domestic sewage and pump mill effluents discharged into water bodies, also can contribute to appearance or increase of NOM concentrations in natural waters[1,2,3].
In their study, Khraisheh et al.[4] presented a simplified interaction process between environment and organic matter. The pathway and main fraction of NOM decomposition are presented in Fig 1.

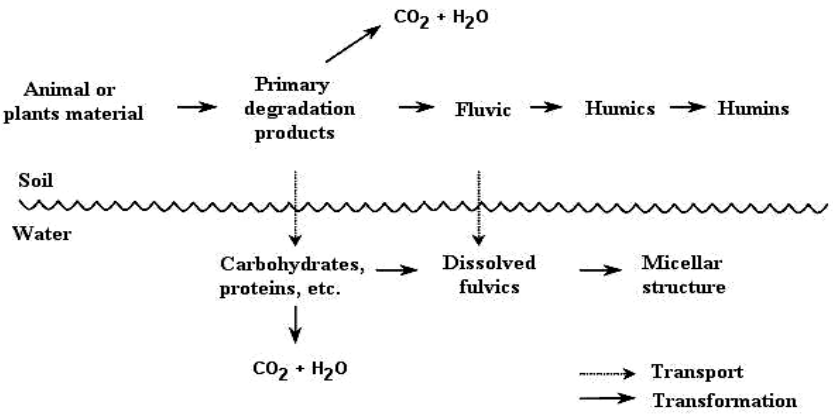

Figure 1. The pathway and main fraction of NOM decomposition[4]

Groundwater and the river Drava water, for the past few decades, were the main sources of drinking water for the population of eastern Croatia. The perennial monitoring of these water sources revealed the increased concentration of natural organic matter[5,6,7].

Fig. 2 shows average values of NOM content, expressed as COD-Mn, in drinking water samples taken from smaller water supply-system in the area of eastern Croatia.

The increased NOM concentration in drinking water causes appearance of yellowish colour and, if water is disinfected with chlorine, formation of carcinogenic trihalomethanes (THM). So, the main goal of drinking water treatment process, in these cases, is to remove NOM from water, since NOM is a primary reactant in the reaction of THM formation. The removal of NOM often implies flocculation, adsorption on activated carbon, oxidation with chemical oxidants or ozone or/and membrane filtration[1]. In the latest decades many scientist also investigated efficiency of so-called advance oxidation processes (AOPs). These processes are based on formation of strong oxidizing species (hydroxyl radical) which oxidize a wide range of organic compounds in water[8].

Since the 80's of the past century, the population of Osijek town has been supplied with drinking water obtained by treatment of groundwater from the water field "Vinogradi". Our latest study of groundwater quality from the water well "Vinogradi" revealed that the groundwater contain high arsenic concentrations which exceed a few time maximum permissible level according to Croatian legislation[9,10]. Before that period, for drinking water production, the Drava river water was pumped and processed and the local Water Company kept a pumping station on the river Drava. Although the NOM concentrations are approximately equal, for the purpose of arsenic concentration reduction, the engineers at Water Company Osijek are considering again using the Drava River as a partial water source. Because of the above mentioned, in this study, groundwater from the water well "Vinogradi" and the Drava river water are investigated in terms of NOM removal by adsorption and determining basic adsorption parameters (adsorbent dosage, pH value and contact time). NOM removal by granulated activated carbon filters could be also an optimal solution for smaller water supply systems (a few thousand inhabitants) whose NOM concentrations are presented in Fig. 2.

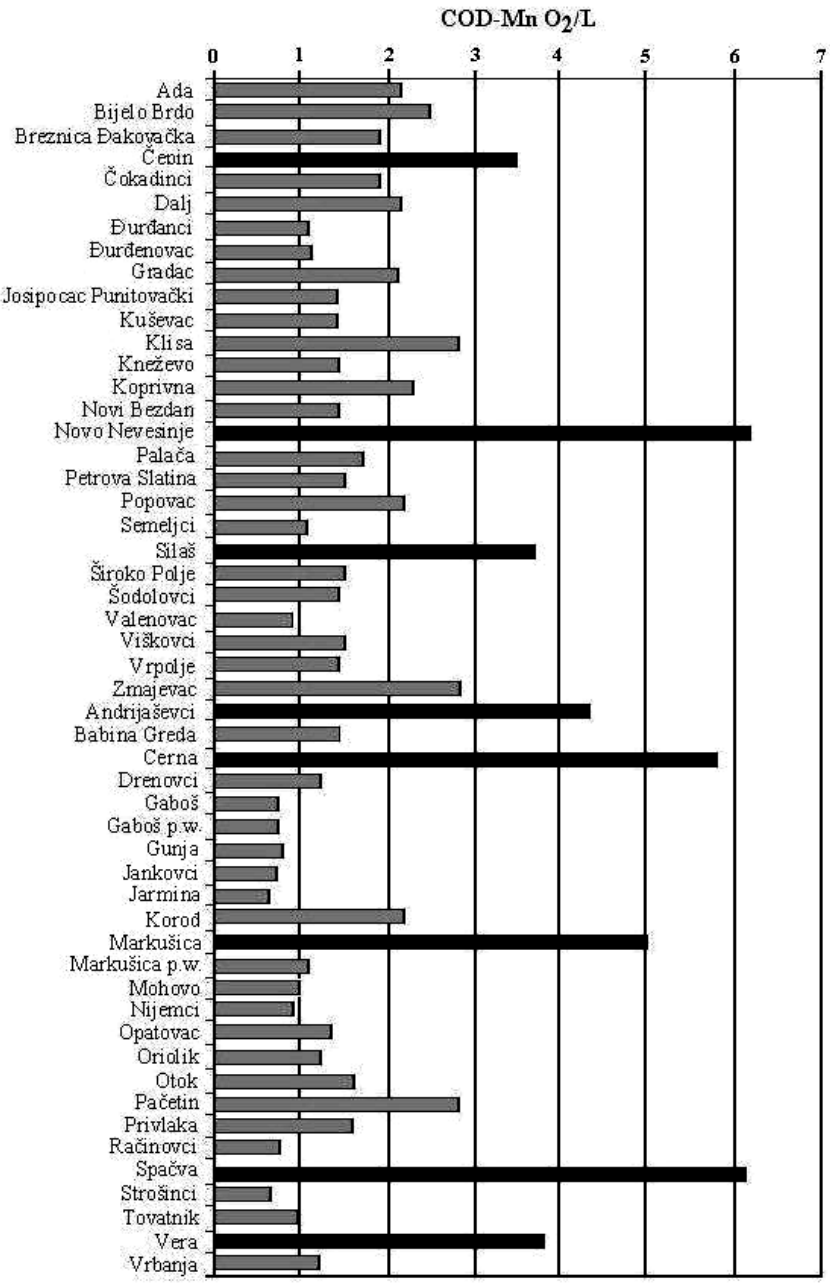

Figure 2. Average values of NOM content in drinking water samples taken from smaller water supply-system in the area of eastern Croatia

MATERIALS AND METHODS

2.1. Natural waters of eastern Croatia

Two types of natural water were examined in this study – aerated groundwater from the Osijek water well "Vinogradi" and the Drava river water. Although groundwater are mainly used to provide drinking water for the population of eastern Croatia, chemical composition e.g. increased concentrations of arsenic, ammonia, manganese and iron have again brought into focus the possibility of processing the Drava river water. The main physical and chemical characteristics of water samples used in this study are presented in Table 1.

Table 1. The physical and chemical characteristics of the Drava river water and groundwater from the water well field "Vinogradi"

Parameters	Units	MPL*	Drava	Vinogradi
Turbidity	° NTU	<4	3,7 - 40	4,38 – 7,20
Colour	° PtCo scale	<20	21 – 85	38 - 70
Odour	-	Without	on algae	on H_2S
Temperature	°C	<25	5,4-24,6	15,5 – 16,5
pH	-	6,5 - 9,5	7,5 - 8,6	7,37 – 7,80
Conductivity	µS cm^{-1}	<2500	280 - 360	850 - 910
Ammonia	mgNH_4^+ /L	<0.50	0,07 - 0,35	1.98 – 2.8
Nitrate	mgNO_3^- /L	<50	4,5 - 8,7	2 - 3
COD-Mn	mgO_2 /L	<5,0	1,46 - 4,2	2,70 – 5,20
total iron	µgFe /L	<200	70 - 220	1100 - 1600
Manganese	µgMn /L	<50	30 – 58	75 - 120
Chlorides	mgCl^- /L	<250	7 -25	4 - 8
Sulphates	mgSO_4^{2-}/L	<250	25 – 45	2 - 4
Hardness	mg$CaCO_3$ L^{-1}	>60	170-210	250 - 330
Phosphates	µg P L^{-1}	<300	18,6 - 40	150 - 350
Oxygen	mgO_2 L^{-1}		6,7-13,8	4,5 – 8,3
Arsenic	µgAs L^{-1}	<10	4,7-12,8	170 - 290

**Maximum permissible level* according to Croatian legislation[10]

2.2. Activated carbons

In this study, four activated carbons were used: Norit Row 0,8 Supra (N), Cullar D (C), Silcarbon K 835 (S) and Hidraffyn 30 N (H).

The type, origin material, particle size, iodine number, total surface area and manufacture names are summarized in Table 2. All carbons are commercially available. Prior to experiments, all activated carbons were washed with deionized water and dried at 105°C for 24 h, cooled and stored in desiccators.

Table 2. Characteristics of used activated carbons

Activated Carbon	Type	Origin material	Particle size (mm)	Manufacturer
Norit Row 0,8 Supra (**N**)	GAC	Peat	0,8	Norit Nederland BV
Cullar D (**C**)	GAC	Coal	0,42 – 1,7	Culligan Int. Company
Silcarbon K 835 (**S**)	GAC	coconut shell	0,5 - 2,5	Silcarbon Aktivkohle GmbH
Hydraffin 30 N (**H**)	GAC	bituminous coal	0,6 – 2,36	Donau Carbon Corporation

2.3. Sorption experiments

The main factors affecting the adsorption process – pH value, contact time and carbon dosage were studied. The test of NOM adsorption onto activated carbon samples were conducted in batch mode. First set of experiments were performed with aerated groundwater from the water well "Vinogradi", while second set were performed with the Drava river water.

The effect of activated carbon dosage on NOM removal was examined using the following dosages 0,05; 0,1; 0,5; 0,7; 1,0 and 2,0 g/l. The specified dosages were put into 100 ml of groundwater or the Drava river water at pH of 7,5 and samples were then agitated 120 rpm in temperature-controlled shaker (Polytest 20, Bioblock Scientific) at 25°C during 180 min.

The effect of pH on NOM adsorption was investigated using 0,1 g activated carbon along with 100 ml of groundwater or the Drava river water, which were put into 200 ml bottles. pH values of groundwater and the Drava river water were adjusted in the range between 4 and 9 before addition of activated carbon using 0,1 M NaOH or 0,1 M HCl.

The impact of contact time on NOM adsorption was studied using 0,1 g activated carbon and 100 ml water sample at pH 7,4 with adsorption times of 15, 30, 60, 120 and 360 minutes, while the impact of carbon dosage on NOM efficiency removal was investigated with carbon dosages between 0,05 and 2,0 g/100 ml. All samples were then agitated 120 rpm in temperature-controlled shaker (Polytest 20, Bioblock Scientific) at 25°C during 180 min.

The removal efficiencies of NOM (%) were determined measuring value of UV absorbance at 245 nm (UV_{254}) e.g. as difference between the initial UV_{254} value (A_0) and UV_{254} value of treated samples (A_e) after filtration using 0,45-μm filter paper (Sartorius vacuum filtration unit).

$$NOM\ removal\ \% = \frac{A_O - A_e}{A_O}$$

To assure the accuracy, reliability and reproducibility of the obtained results, each experiment was carried out in duplicate and average values only are reported.

2.4. Analytical methods

Determination of pH was made using pH-meter Seven Easy (Metler Toledo) while UV absorbance at 254 nm of initial and treated samples was measured by UV-Vis spectrophotometer Specord 200 PC (Analytik Jena AG) with 1 cm length quartz cell after 0,45 μm pore size membrane filtration according to the

procedure described in the Standard Methods for Examination of Water and Wastewater[11].

RESULT AND DISCUSSION

3.1. Effect of activated carbon dosage

The effect of activated carbon dosage on NOM removal was investigated in the range of dosages from 0,05 - 2,0 g/l and the results are shown in Figure 3. As it was expected, the NOM removal efficiency was increased with amount of activated carbon in both experimental sets, although a linear increase of activated carbon dosage did not linearly increased amount of NOM removal.

Figure 3 shows significant differences of NOM adsorbed onto activated carbons at the highest dosage (2 g/l) and at dosages up to 0.7 g/l, results are similar. The highest amount of NOM (61%) was removed from aerated groundwater when Hydrafiin 30 N was used in 2 mg/100ml dosage, while the lowest removal at the same dosage was obtained using Silcarbon K 835 (44%). It is also clearly visible in Fig. 3B that three activated carbons (Norit Row 0,8 Supra (N), Cullar D (C) and Silcarbon K 835) gave approximately the same results when NOM were removed from the Drava river water. In this set, applications of Hidraffyn 30 N also gained the highest NOM removal. Lin and Zhan[12] in study of humic acid adsorption onto modified zeolite also observed non-linear relationship between humic acid adsorbed and increase of adsorbent dosage.

Figure 3 shows that NOM removal by adsorption onto activated carbons is more effective when NOM was removed from groundwater rather than the Drava river water. Relationship between NOM removal and water hardness was also determined by Gorenflo et al.[13]. They suggested water hardness, e.g. higher concentration of calcium ions in raw water presumably complex with humic substances which results with generally better NOM removal from hard waters.

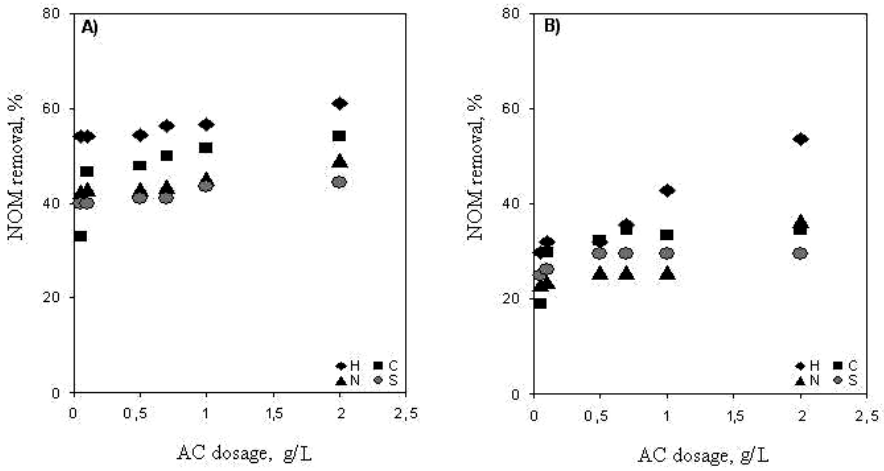

Figure 3. Effect of activated carbons dosage on the NOM removal from A) aerated groundwater and B) Drava river water

3.2. Effect of pH

The effect of pH on the NOM removal was studied in the range 4,0 – 9,0 at the temperature of 25°C and contact time was 120 min. Dosage of activated carbon of 0,1 g/l was chosen for all adsorption

experiments. The result of the effect of pH on NOM removal from groundwater samples is shown in Fig. 4A, while the effect of pH on NOM removal from the Drava river water samples is shown in Fig. 4B.

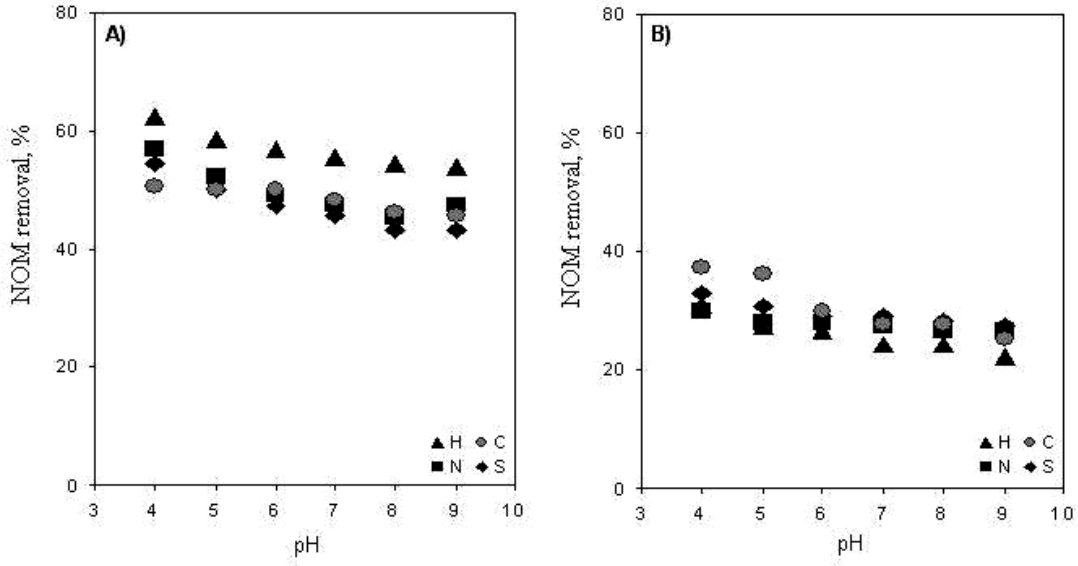

Figure 4. Effect of pH on the NOM removal from A) aerated groundwater and B) Drava river water

In both sets of experiments the percentage of NOM removal decreased with increase of pH. In the first set of experiments, when groundwater samples were used (Fig. 4A), maximum of NOM removal occurred at pH 4.0. The largest amount of NOM (62%) was removed at pH 4.0 when AC Hydraffin 30 N was used, while the lowest NOM removal (50%) was obtained using AC Cullar D. In the second set of experiments (Drava river water) significantly less NOM removal was detected. The best result of NOM removal (37%, pH 4,0) was noted when Cullar D was applied and the lowest NOM adsorption was on Norit Row 0,8 Supra (29%). In both sets of experiments, it can be observed that the weakest effect of pH increase on NOM removal is detected at AC which showed the lowest capacity of NOM adsorption (groundwater- Cullar D; Drava river water – Norit Row 0,8 Supra). Similar effect of pH on NOM adsorption has been previously observed and explained by many authors[14,15,16] who generally claim that the adsorption amounts of some anionic adsorbates on the adsorbents with positive surface charges often show a trend of decrease with pH increasing due to less attractive or more repulsive electrostatic interaction at higher pH values.

3.3. Effect of contact time

Figure 5 shows the effect of contact time on the NOM removal efficiency from aerated groundwater (A) and the Drava river water (B) by followed activated carbons: Norit Row 0,8 Supra (N), Cullar D (C), Silcarbon K 835 (S) and Hidraffyn 30 N (H).

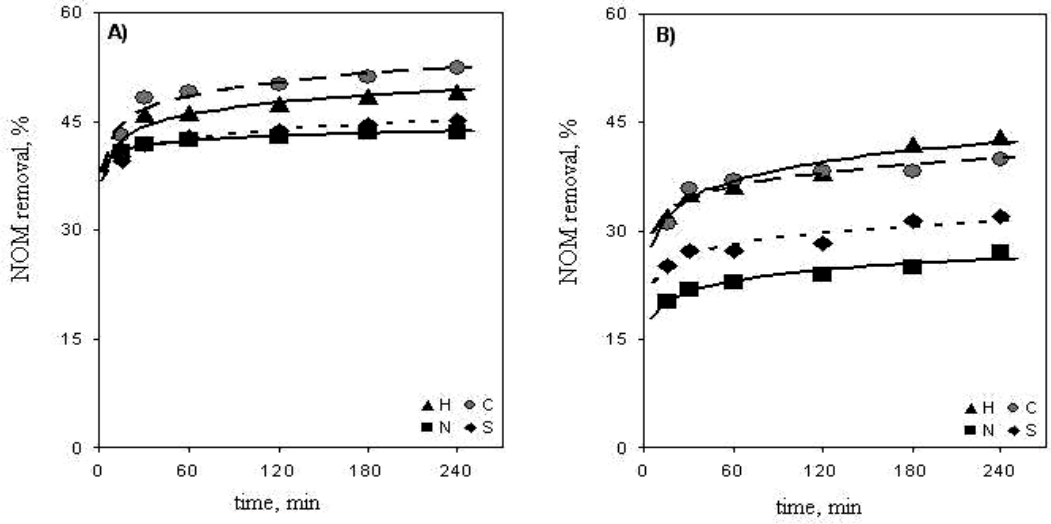

Figure 5. Effect of contact time on NOM removal from A) aerated groundwaterand B) Drava river water

It can be seen in Figure 5 that a percentage of NOM removal increased with time in all experiment sets. The highest percentages of NOM removal and near-equilibrium phase were obtained during longest contact period (240 min). When aerated groundwater samples were used, all four activated carbons showed similar efficiency. The highest removal and the most positive effect of contact time on NOM removal was at AC Cullar D (52% within 240 min) and the lowest removal and negligible effect of contact time was detected at Norit Row 0,8 Supra (Fig. 5A). The effect of contact time on NOM removal from the Drava river water is shown in Fig. 5B. In Fig. 5B it can be observed that longer contact time had strongest impact on NOM removal by Hydraffin 30 N and Cullar D, while no significant effect of contact time was observed when Silcarbon K 835 and Norit Row 0,8 Supra were used. A previous study of NOM adsorption onto granular activated carbons, Zhang and Bai[15] obtained similar results. The aforementioned authors investigated NOM adsorption kinetics within 4000 minutes at similar pH values and their results showed fast NOM adsorption within first hours of experiments.

CONCLUSION

The effects of activated carbon dosage, pH value and contact time on NOM removal from natural waters (groundwater and Drava river water) using four granulated activated carbons (Norit Row 0,8 Supra, Cullar D, Silcarbon K 835 and Hidraffyn 30 N) were studied. First set of experiments were performed with the aerated groundwater from the water well "Vinogradi" while second set were performed with the Drava river water. The NOM removal efficiencies of all four activated carbons were found to increase with increasing activated carbon dosage. The highest percentages of NOM removal were achieved when dosage of 2 g/l activated carbon Hidraffyn 30 N were used in both sets of experiment (61% for groundwater, 53% for Drava river water). When pH effect of water was investigated

in the range from 4 to 9, four tested activated carbon showed similar decrease of NOM removal. Variation of pH value had weakest impact on active carbon Cullar D in first set and Norit Row 0,8 Supra in the second set. Highest amount of NOM was removed from the groundwater with Hidraffyn 30 N (62%) at pH 4, and from the Drava river water with Cullar D (37%) at the same pH value. The effect of contact time also had similar influence on four tested activated carbons. The obtained results indicate that portion of NOM adsorbed onto activated carbons is rapidly increased within 60 minutes and after that period, amounts of NOM removed are approximately equal.

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EFFECT OF POULTRY MANURE ADDITION ON THE AEROBIC COMPOSTING PROCESS OF ORGANIC FRACTION OF MUNICIPAL SOLID WASTE

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

The aerobic composting of organic fraction of municipal solid waste (OFMSW) with addition of poultry manure (PM) was conducted in order to determine the influence of manure on process parameters (temperature, pH, electrical conductivity, moisture content, oxygen concentration, carbon dioxide concentration, and organic matter content). The experiment was carried out with three different mixture ratios, in three laboratory scale reactors (35 litres), specially designed for this purpose, with a forced aeration, daily mixed to keep homogeneity of the mixtures, during 22 days of composting. The results showed that addition of poultry manure has a great impact on the dynamics of aerobic composting process of OFMSW. According to the results, the mixture in reactor 2 (50% OFMSW, 33.33% poultry manure, 8.33% sawdust and 8.33% mature compost) showed best conditions during and at the end of the process. Therefore, this mixture is recommended for composting process. For this mixture the following results have been achieved: maximum temperature of 65°C, final pH value of 8.02, final EC value of 1.398 dS/m, final moisture content of 61.43%, minimum O_2 concentration of 3.88% (vol.) and maximum CO_2 concentration of 16.98% (vol.), final loss of organic matter content of 63.04%.

Key words: composting, poultry manure, organic fraction, municipal solid waste, laboratory reactor.

INTRODUCTION

Disposal of the municipal and other wastes in Tuzla as well as in other cities represents a great problem. The capacity of landfills is not unlimited, which is the case with the landfill "Desetine", so it is necessary to seek and use alternative methods to waste disposal. Such methods are: recycling, energy production by incineration (heat and electric energy), usage at construction sites, composting organic fraction etc. According to assessment in Bosnia and Herzegovina, approximately 562,100 tons of waste is daily generated from households, 40% of that is organic fraction which is potential for composting[1]. Also a large part of the municipal waste can be found on illegal landfills, so this waste is rather not accessible. The advantages of composting are: reduction of waste amount and GHG emission for landfills, loss of nutrients, etc. The Council Directive (1999/31/EC)[2] obliges Member States to reduce the amount of biodegradable waste that they

by applying the best available techniques (BAT), composting is one of these techniques.

It is common to perform co-composting organic fraction of municipal solid waste (OFMSW) with other organic wastes. Such materials should be rich in nitrogen. Among these materials, poultry manure (PM) is considered appropriate[3]. By adding poultry manure, it is possible to optimize C/N ratio of OFMSW[4]. Thereby, desired or nearly desired C/N (25-50:1)[5] can be made for better performance of composting process. Besides C/N, the poultry manure will optimize pH, moisture content, final content of organic matter, etc.

The main aim of this study was to determine the influence of addition of poultry manure on dynamics of aerobic composting of organic fraction of municipal solid waste, as well as to determine which of three mixtures should be recommended for composting process.

MATERIALS AND METHODS

Composting materials

The materials used in this experiment were: organic fraction of municipal solid waste (OFMSW), poultry manure (PM), sawdust and mature compost.

OFMSW (collected from local markets, offices, local parks and student restaurants) consisted of: food leftovers, vegetable and fruit wastes (plums, apples, grapes, pears, oranges, peaches, cabbage, potato, eggplant, tomato, pepper and etc.), yard waste (trimmed grass, fallen leafs and small branches), office and newspaper, cardboard. Paper and cardboard was cut into 3 – 5 cm pieces. In the preparation of OFMSW, materials were cut or mashed and well mixed to achieve better homogeneity. Final composition was: food, vegetables and fruit 56.52%, paper and cardboard 30.43% and yard waste 13.04%.

PM was brought fresh and was added to the mixture of OFMSW to optimize C/N. Sawdust was used as bulking agent to improve aeration and as an additional source of carbon. Mature compost was used to speed up the process. The physical–chemical characteristics of all materials for composting, measured and calculated in laboratory before starting the experiment, are presented in Table 1.

Table 1. Physical–chemical characteristics of raw materials

Raw material	Dry matter (% d.b.)	Moisture content (% w.b.)	Organic matter (% d.b.)	Ash (% d.b.)	pH	EC (dS/m)	C (%)	N (%)	C/N
OFMSW	40.17	59.83	91.69	8.31	4.98	1.19	50.94	0.66	77.18
PM	28.97	71.03	78.89	21.11	8.31	3.77	43.83	5.02	8.73
Sawdust	89.97	10.03	99.90	0.10	5.31	0.24	55.50	0.28	198.21
Mature compost	67.49	32.51	40.31	59.69	6.92	0.35	22.39	1.21	18.5

w.b. – wet basis d.b. – dry basis

Experimental setup and procedure

The experiment was conducted in three laboratory scale reactors with a volume of 35 litres (height 55 and diameter 36 cm), made from stainless steel. Reactors were coated with 10 mm thick polyethylene foam for heat insulation. Through the centre of lid vertical rotating axis with blades, for mixing, was attached on perforated plate at bottom of the reactor. Through the plate liquids from mixture were filtrated. On the lid were pipes for: air inlet via compressor (Trudbenik, B&H), outlet gasses and a pipe for gas sampling. Second hole on lid was for thermocouple (type T. Digi – Sense, Cole –Palmer, USA), for temperature measurement in the centre of mixture every 15 minutes. Before they were introduced to the atmosphere, outlet gasses were washed with NaOH (1M) and H_3BO_3 (0.65M), in order to remove the traces of ammonia and carbon dioxide. The experimental set-up is shown in Figure 1.

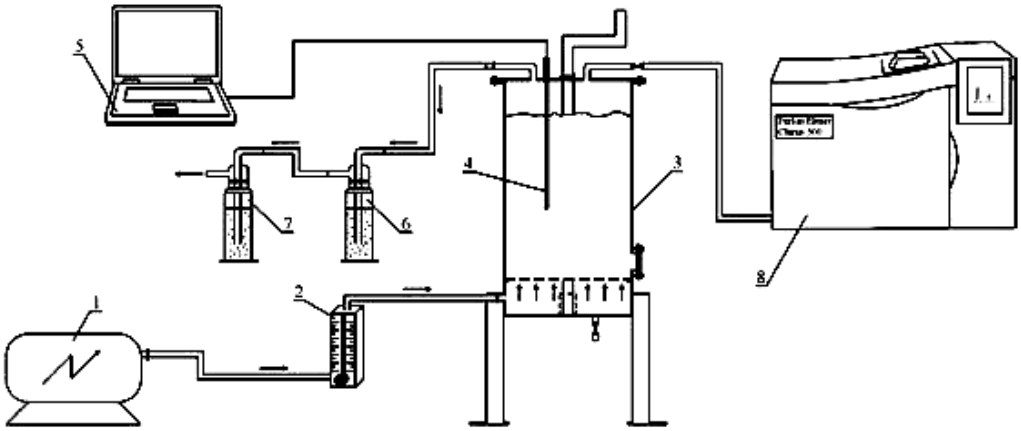

Figure 1. Schematic diagram of reactor system: 1. compressor, 2. airflow meter, 3. reactor, 4. thermocouple, 5. laptop, 6,7. gas washing bottles (NaOH, H_3BO_3), 8. gas chromatograph

Sampling and analysis

During the daily experiment, approximately at the same time, samples were gathered, 25 – 30 grams, after mixing from different locations in a reactor. Each analysis was done in triplicate with calculation of the mean value. The analysis was conducted immediately after sampling.

The concentrations of oxygen and carbon dioxide were measured using gas chromatograph (Gas Chromatograph Clarus 500 – PerkinElmer ARNEL, India) and software TotalChrom Workstation.

The moisture content was determined by drying sample in an oven at 105°C for 24h[7]. The organic matter content was analyzed by burning the sample in the oven at 550°C for 6h[7]. The loss (conversion) of organic matter was calculated from initial and final content of organic matter, using the equation[6]:

$$k = \frac{\{[OM_p(\%) - OM_k(\%)] \cdot 100\}}{OM_p(\%) \cdot [100 - OM_k(\%)]} \cdot 100 \quad (1)$$

Where are:

- OM_p – beginning organic matter content (%);
- OM_k – final organic matter content (%);
- k – loss of organic matter content (%).

The nitrogen content (%N) was calculated using the standard method [7]. The content of carbon (%C) was calculated from the content of inorganic matter (ash) (%), according to the equation[6]:

$$\%C = \frac{(100 - \%NM) \cdot 100}{1.8} \qquad (2)$$

Electrical conductivity (EC) and pH were measured in aqueous extract of sample, dissolved with distilled water and mixed again with distilled water in the ratio 1:10. The suspension was filtrated through Whatman 42 filter paper. pH and EC measurements were carried out using PC 5100 pH/Conductivity meter (Oakton, Singapore), with two separated electrodes.

Mixture preparation

The composition of the reactor mixtures are given in Table 2. The mixtures were prepared in the following way: food, fruit and vegetable waste were chopped and mashed, then mixed together in plastic tubs, afterwards were added paper, cardboard and yard waste, and mixed again. PM, sawdust and mature compost were added at the end. The mixture was again very well remixed to achieve homogeneity. Initial physical-chemical characteristics of mixtures are given in Table 3.

Table 2. Percentage composition of mixtures in the reactors

Reactor	OFMSW (%)	Manure (%)	Sawdust (%)	Mature compost (%)
R1	60	20	10	10
R2	50	33.33	8.33	8.33
R3	42.86	42.86	7.14	7.14

Table 3. Initial physical–chemical characteristics of mixtures

Reactor	Moisture (%)	Organic matter (%)	pH	EC (dS/m)	C (%)	N (%)	C/N
R1	45.56	87.53	5.17	1.501	48.63	1.21	40.19
R2	58.17	85.61	6.27	2.169	47.56	1.30	36.59
R3	60.38	83.39	6.37	2.627	46.61	1.38	33.77

Statistical analysis

Statistical analysis was performed using ANOVA analysis (“Multiple Range Test” – variance analysis) in statistical software Statgraphics Plus 5.1 (Statistical Graphic Corp., 2011, USA).

RESULTS AND DISCUSSION

Temperature profiles

Immediately after filling the reactors, temperature rise was observed (Figure 2). The reason was addition of the mature

compost and huge amount of microorganism in mixtures that accelerated decomposition of the organic matter. In reactors 2 and 3 which had greater amount of the PM, maximum temperatures were 65°C and 70°C respectively, while in reactor 1 with smaller amount the temperature was 61°C. Temperature maintained above 55°C for 2 days, in which the sanitation was achieved[3,9]. After temperature drop in the reactor 1, rise in the temperature occurred again from 7th until 18th day. The same happened in reactor 2 on 16th day. This observation can be explained by replenishment of oxygen supply for metabolism of microorganisms[8] by presence of cellulose (sawdust, paper and cardboard), which starts to decompose after easily degradable components are decomposed[9,10]. This had impact on final values of moisture content and organic matter. The results did not differ a lot from results of other experiments in which PM was used, and also in which OFMSW was composted without any additives[10,11,12,13,14]. Slower rise of temperature was noticed compare to other experiments which used PM[5,9,15,16,17,18]. There were statistically significant differences in temperature regime between reactors 1 and 2 (P<0.05).

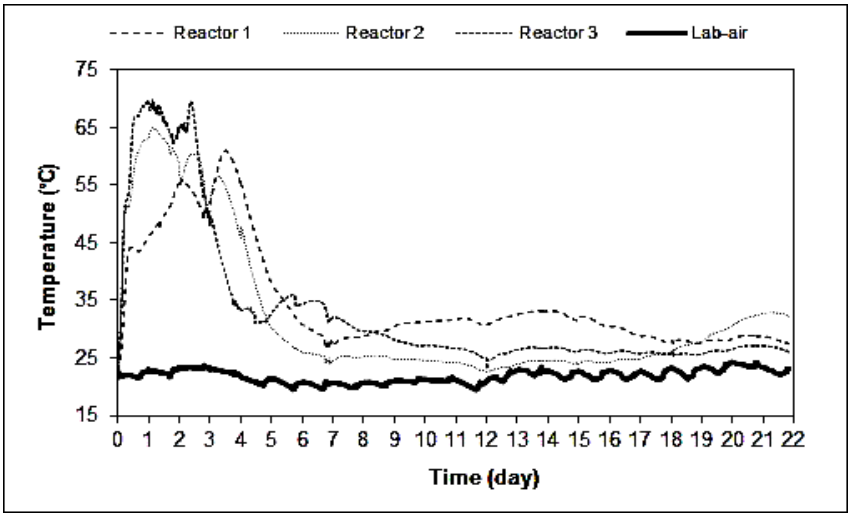

Figure 2. Changes of reactor and ambient temperatures

pH

According to the recommendations for thermophilic composting[19], the pH value was satisfactory in all three reactors. Changes in pH are shown in Figure 3. Maximum values in reactors 1, 2 and 3 were 7.99, 8.71, and 8.75, and at the end 7.62, 8.02 and 8.75, respectively. These values were between 6 and 8.5, which is acceptable for great number of plants. Alsosuch raw compost is applicable on soils with unchanged alkali. Relative low value was due to rottenness of fruit and vegetables, but the increase in pH occurred due to degradation of proteins and formation of ammonia[19,20,21,22]. Similar changes in pH were observed in composting of different materials that did not contain manures[10,12,17,23]. It was observed that there were significant differences in pH between reactor 1 and reactors 2 and 3 (P<0.05).

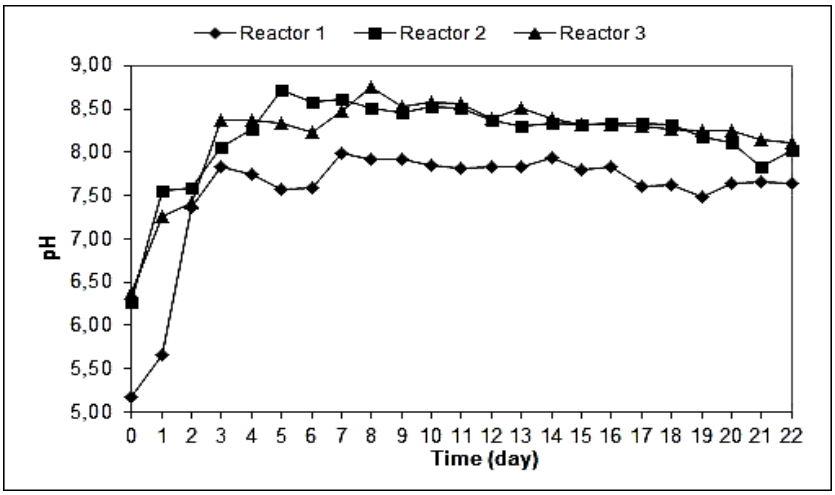

Figure 3. Changes of pH during the process

Electric conductivity (EC)

The PM increases EC in composting mixture[7]. High EC values (1.501, 2.169 and 2.627 dS/m) dropped to the 0.774, 1.398 and 1.830 dS/m at the end of the process (Figure 4). Final low values should not have any negative effect on the soil and plants[10], which is confirmed by study of Hachica et al.[12]. The decomposition of organic matter had good impact on reduction of EC, although PM was rich in minerals[13]. These raw materials are adequate for composting process that would not poison or inhibit plant growth. It was observed that there were significant differences in EC between all three reactors ($P<0.05$).

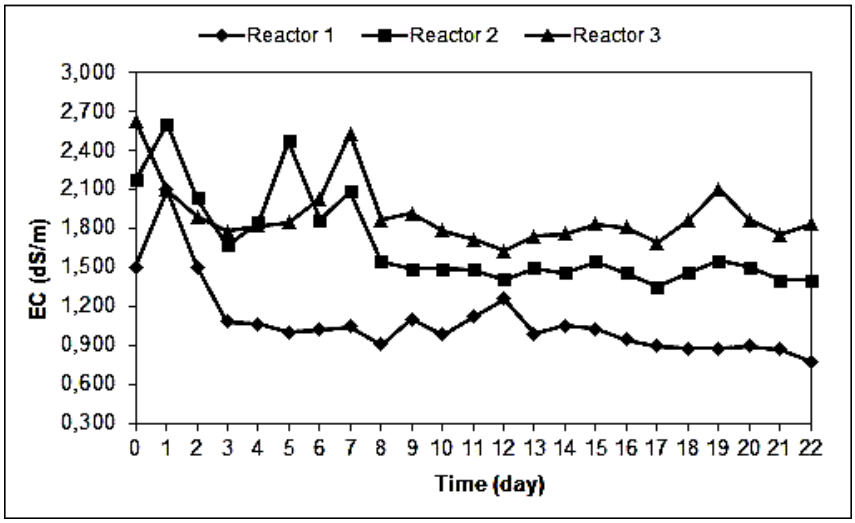

Figure 4. Changes of EC during the process

Moisture content

The initial moisture contents in reactors (45.66%, 58.17% and 60.38%) were within recommended values for the composting of municipal solid waste [8,24,25,26]. Also, final moisture contents were within the values for rendering and maintaining thermophilic

profiles which fulfilled the condition of moisture influence on temperature[19]. Changes in moisture content are shown in Figure 5. The values of MC at the end of process in reactors 1 and 2 were higher than initial ones, 65.14% and 61.43% respectively, while in reactor 3 they decreased to 57.32%. The reason for increase in moisture content was probably in the secondary decomposition of organic matter after 7th and 18th day. There were statistically important differences between moisture content values in all three reactors ($P<0.05$).

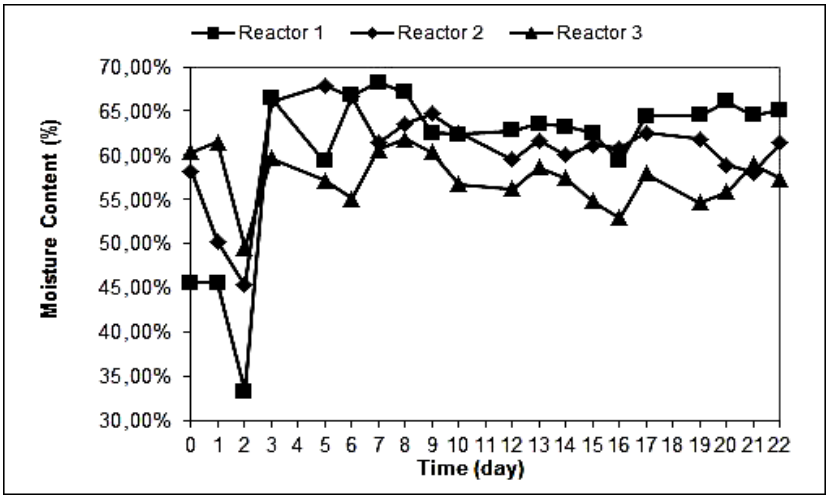

Figure 5. Changes of moisture content during the process

The concentrations of oxygen and carbon dioxide

The changes in CO_2 and O_2 concentrations (Figures 6 and 7)followed changes in temperature. At the highest temperatures, O_2 concentrations were 8.96%, 3.88% and 1.41%, and concentrations of CO_2 were 13.02%, 16.98% and 20.38%. All of these concentrations were when the highest loss of organic matter occurred. After achieving the lowest concentration of O_2 and the highest concentration of CO_2, concentrations started to change, probably due to stabilization of the compost[12].

Changes were recorded in reactors 1 and 2 after 8th and 17th day, respectively, with the changes in temperature. Reason for that lies in a microbial activity, decomposition of cellulose matter and in replenishment of oxygen supply after mixing. Similar results were also found[23]. The mean values of O_2 in reactors were close to values recommended for aerobic composting[17]. Therefore, the process was conducted in the aerobic environment[27]. Statistical analysis showed that there were significant differences in CO_2 and O_2 concentrations between reactors 1 and 2 ($P<0.05$).

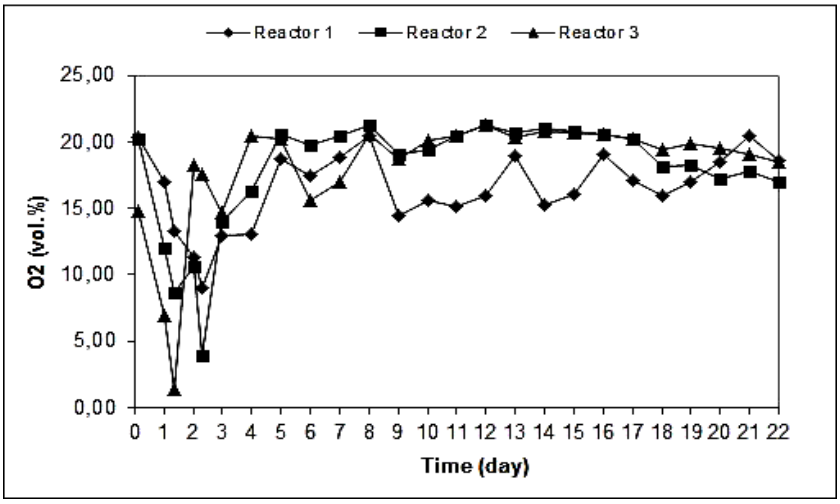

Figure 6. Changes of O_2 concentration during the process

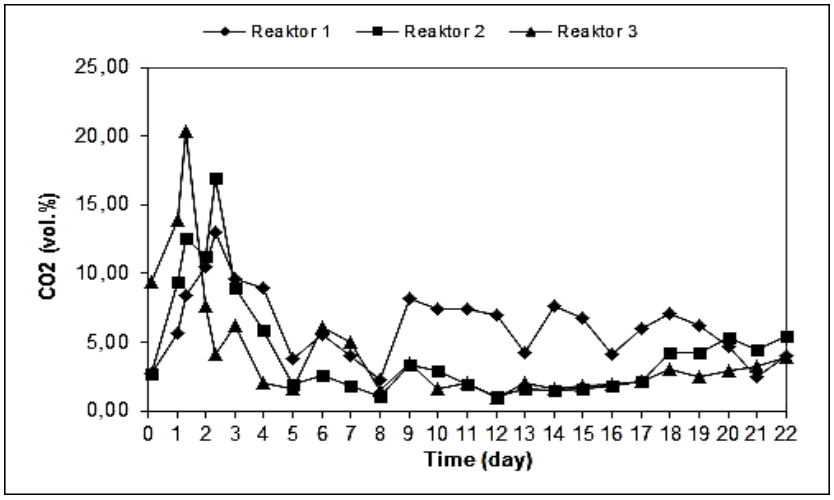

Figure 7. Changes of CO_2 concentration during the process

Organic matter

The amount of organic matter which has degraded depends on an amount of organic carbon[8]. Initial organic matter contents were 87.53%, 85.61% and 83.89%, and organic matter contents after 22 days were 67.20%, 68.74% and 72.35% (Figure 8). There were statistically significant differences between organic matter content for all reactors (P<0.05). Loss of matter in reactor 1 was 70.81%, which contained the least amount of PM. Smaller losses were recorded in the reactors with greater amount of PM[4]. In reactors 2 and 3 loss of organic matter content were 63.04% and 49.75%, respectively (Figure 9). The greater amount of OFMSW with sawdust over PM served well in organic matter loss[16,28] High values for loss of organic matter content are due to lack of impurities which can be found in municipal solid waste[29]. It can be assumed that loss of organic matter during the maturation phase should be almost the same, as it was at the end of process[23].

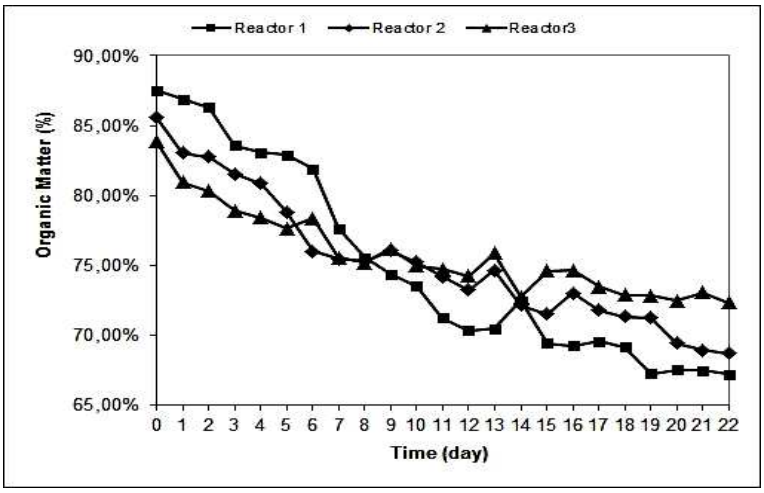

Figure 8. Changes of organic matter content during the process

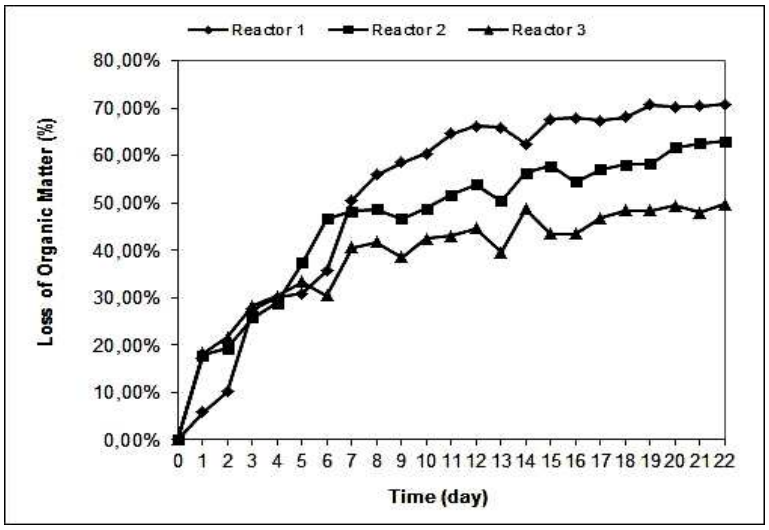

Figure 9. The loss of organic matter content during the process

CONCLUSIONS

The addition of poultry manure has a great impact on the dynamics of aerobic composting process of OFMSW, which was confirmed by changes of temperature, pH and EC values, concentrations of O_2 and CO_2, moisture content and organic matter content. The mixtures of OFMSW and PM had achieved thermophilic conditions in all three reactors. According to the results, the mixture in reactor 2 (50% OFMSW, 33.33% poultry manure, 8.33% sawdust and 8.33% mature compost) showed best conditions during and at the end of the process. Therefore, this mixture is recommended for composting process. pH and EC values at the end of process indicate that this compost is applicable on soil and many plants. Before possible application of compost, the content of heavy metals should be determined. Although the mixtures were not additionally moisturized, the moisture content until the end of process was within desirable values. Decrease of O_2 concentration and increase of CO_2 concentration followed temperature changes and organic matter degradation. The further study should be focused on

improvement of mixing and aeration, as well as exploring of different ratio of the same and different materials.

Acknowledgements

The research conducted and presented within the study was a part of Research Project „Possibilities for the application of composting process of municipal solid waste with different additives in a reactor system“, financially supported by the Federal Ministry of Education and Science of Bosnia and Herzegovina.

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TOTAL PHENOLS CONTENT, ANTIOXIDANT ACTIVITY AND COLOUR OF WHEAT BREAD WITH ADDITION BUCKWHEAT FLOUR

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

Wheat bread was prepared by replacing 15%, 30% and 40% of white wheat flour with wholegrain buckwheat flour. Total phenols content (Folin–Ciocalteu method) and antioxidant activity (FRAP) of buckwheat flour enriched wheat bread samples and flour mixtures were analysed and compared with wheat bread and wheat flour (control samples). Wholegrain buckwheat flour (17.91 mg GA/L extract) contained higher total phenols than wheat flour (9.11 mg GA/L extract). The incorporation of buckwheat flour in bread samples increased the total phenols content from 7.46 (mg GA/L extract) to 10.13 (mg GA/L extract), and antioxidant activity from 231.45 (µmol Fe^{2+}/L extract) to 368.45 (µmol Fe^{2+}/L extract). Total phenols content decreased during the baking process, while the antioxidant activity increased. Bread samples with 15%, 30% and 40% of wholegrain buckwheat flour showed lower *L* values of crust and crumb colour compared to the control sample.

Key words: buckwheat bread, colour, total phenols, antioxidant activity

INTRODUCTION

Bread and bakery products have an important role in human nutrition. Generally, wheat bread is a good source of irreplaceable nutrients and energy for the human body. The most common bakery products are obtained from white flour, but with increasing awareness of the importance of proper nutrition and healthy lifestyle grow need for products that have improved nutritional composition with potentially preventive effects on health. Demand for products with improved functional properties is expressed in the bakery industry[1]. There are many special types of bread in the category of functional products that are rich in minerals, dietary fibre, vitamins, inulin, oligosaccharides, omega-3 fatty acids, β-glucans, flax seeds and others. Buckwheat is pseudocereal which does not contain gluten and is mainly used in the production of gluten-free products[2]. Buckwheat is a rich source of proteins, carbohydrates, minerals, fibre, flavonoids, and other compounds which are to participate in lowering blood pressure, reduce cholesterol levels, blood glucose control and prevention of cancer[3-6]. Unlike to wheat and other cereals, buckwheat proteins have a good balanced amino acids composition and contain all of essential amino acids[7], although their digestibility is relatively low[8,9]. Also buckwheat contains phenolic compounds which show significant antioxidant

activity[10,11]. Rutin and quercetin are the main antioxidants in buckwheat that prevents lipid peroxidation and activity of free radicals[12,13]. Compared to antioxidant activity of frequently used cereals, buckwheat possesses higher antioxidant activity, mainly due to high rutin content[13,14]. Wholegrain wheat and wholegrain buckwheat flours contain more phenols compounds and show greater antioxidant activity compared with refined wheat and buckwheat flour[15]. The aim of this work was to investigate influence of wholegrain buckwheat flour on the total phenols content, antioxidant activity and colour of wheat bread.

MATERIALS AND METHODS

Materials

The ingredients used in the bread samples making were wholegrain buckwheat flour (WBF), white wheat flour (WF), and salt and dry bakery's yeast. All ingredients were purchased from local market. All chemicals and reagents were purchased from: Fluka, Switzerland (2,4,6-tri[2-pyridyl]-s-triazine); Merck, Germany (gallic acid) and Semikem, Sarajevo (Folin-Ciocalteu's reagent, chloric acid 37% p.a.; ferrous sulphate heptahydrate; ferric chloride hexahydrate; sodium acetate trihydrate; acetic acid; sodium carbonate). The ingredients for bread samples making were dosed according to the formula in Table 1.

Table 1. The formulations of bread samples (% on the flour basis)

Ingredients	Bread samples			
Wheat flour (%)	100	85	70	60
WBF (%)	-	15	30	40
Salt (%)	2	2	2	2
Yeast (%)	3.5	3.5	3.5	3.5
*Water (%)	58	59	60	60.5

*according to water absorption (farinograph test)

Bread making

Bread making was conducted under the following conditions: mixing of ingredients to form dough (11 minutes), dough resting (15 minutes, dough temperature 27°C), manual remixing, intermediate fermentation (15 minutes), dough dividing into dough pieces (470 g), shaping the dough into a loaf, final proofing (60 minutes, 35 ± 2°C, 75% relative humidity), baking at 240°C (23 ± 1 minutes). After the baking, the bread samples were cooled at room temperature and packaged in plastic bags. Some of samples were stored at -22 °C for analysis of total phenol and antioxidant activity after 30 days.

Chemical composition of flour

The composition of flour samples, including moisture content, ash, crude protein and total fat content, were determined according to ICC Standard Methods[16]. All the measurements were taken in triplicate and expressed as mean value ± standard deviation (SD).

Sample preparation and extraction

In all samples of bread and flour, total phenol was extracted with water. Pieces of the crumb (50 g) and the crust (50 g) were taken from various parts of the bread, air-dried and milled together in a kitchen electric mill. 5 g of powder sample was taken for extraction and diluted in 100 mL distilled water. The content was mixed from time to time for 60 minutes at room temperature (20°C), and then filtered through filter paper (S&S 589 Black ribbon). Extraction of phenol from 5 g flour sample was done according to the same procedure as for the powder bread samples. The extract obtained by this procedure was used for determination of antioxidant activity and total phenols content.

Determination of total phenols (TP) content

Total phenols in water extracts of flour and bread were determined with Folin-Ciocalteu reagent as described by Singleton et al.[17]. The extract (200 μL) was mixed with 2 mL of Folin-Ciocalteu reagent (previously diluted 10 times with distilled water). After 5 minutes, 1.8 mL sodium bicarbonate solution (7.5% w/v) was added to the mixture and after incubation for 120 min at room temperature, the absorbance was measured at 765 nm using a UV/VIS spectrophotometer (UV mini 1240, Shimadzu). The concentration of total phenols compounds in extracts was determined as gallic acid equivalent (GA) using an equation obtained from a standard gallic acid graph. Results are expressed as mg GA/L of extract. All the measurements were taken in duplicate and expressed as mean value ± standard deviation (SD).

Determination of total antioxidant activity

The antioxidant activity of aqueous extracts of flour and bread was determined by FRAP (Ferric Reducing Antioxidant Power) method[18]. FRAP reagent was prepared by mixing TPTZ (2, 4, 6-tripyridyl-s-triazine) solution (10 mM TPTZ solution was prepared in 10 mL of 40 mM HCl), 20 mM $FeCl_3 \cdot 6H_2O$ and acetate buffer (0.3 mol/L, pH 3.6) in the ratio 1:1:10. All solutions were used on the day of preparation. FRAP reagent was prepared and thermostated at 37°C. A volume of 200 μL extract was mixed with 1.8 mL of FRAP reagent and the absorbance of the reaction mixture was measured at 593 nm (UV mini 1240, Shimadzu) after incubation at 37°C for 10 min. The antioxidant activity was determined as μmol Fe^{2+}/L of extract using an equation obtained from a standard curve. Standard curve was prepared using different concentrations (50-1000 μmol/L) of $FeSO_4 \cdot 7H_2O$ and absorbance were measured as sample solution. All the measurements were taken in duplicate and expressed as mean value ± standard deviation (SD).

Instrumental colour measurement of bread samples

The colour measurements were made with a colorimeter Croma Meter CR 300 (Konica Minolta, Japan) according to the method CIE 1976 - Lab Colour Space. A standard white plate was used to standardise the instrument. Colour of the crumb and crust of bread is expressed in

the CIE-Lab parameters as *L* (white/black), *a* (red/green) and *b* (yellow/blue). Results are presented as the mean value of five measurements ± standard deviation (SD). Whiteness index (WI) was calculated based on the following equation[19]: $WI = 100 - [(100 - L)^2 + a^2 + b^2]^{1/2}$

Statistical analyses

One-way analysis of variance (ANOVA) and multiple comparisons (Duncan's *post-hoc* test) were used to evaluate the significant difference of the data at $p < 0.05$. Pearson's correlation coefficient (r) was used for the analysis of linear correlation between total phenols, FRAP and *L*, *a* and *b* colour values. Two-way t-test (2-tailed) was used to test the statistical significance of the correlation coefficient ($p < 0.05$). Data were analysed using the software package SPSS V.15.

RESULTS AND DISCUSSION

The analysis of wheat and buckwheat flour used for bread production showed that buckwheat flour has lower water content, while mineral and fat content is higher compared to wheat flour (Table 2). These results have been expected, having in mind that we used WBF for the experiment. This is why mixed bread flour containing 15%, 30% and 40% WBF has a considerably increased mineral content (between 0.83% and 1.28%) compared to wheat flour (0.49%).

Table 2. Chemical composition of flour samples

Flour samples	Water (%)	Ash (%d.m.)	Proteins (%d.m.)	Fats (%d.m.)
WF	13.40 ± 0.13	0.49 ± 0.03	11.69 ± 0.55	1.03 ± 0.16
WF + WBF (85 % + 15 %)	12.86 ± 0.03	0.83 ± 0.05	11.81 ± 0.12	1.23 ± 0.09
WF + WBF (70 % + 30 %)	12.09 ± 0.17	1.13 ± 0.09	11.9 ± 0.23	1.5 ± 0.04
WF + WBF (60 % + 40 %)	11.58 ± 0.10	1.28 ± 0.06	12.01 ± 0.11	1.62 ± 0.07
WBF	10.15 ± 0.04	2.4 ± 0.05	12.4 ± 0.22	2.5% ± 0.01

*WF – wheat flour; WBF – wholegrain buckwheat flour

The results obtained by determination of total phenol content in water extract of flour samples (Table 3), show that total phenol concentration in WBF (17.91 mg GA/L extract) is almost twice as high compared to wheat flour (9.11 mg GA/L extract). There is a statistically significant correlation between the share of WBF and total phenol content ($r = 0.969$; $p \leq 0.01$). The results of this analysis confirm the results published by several other authors[13,15].

Table 3. Total phenols (TP) contents and antioxidant activity (FRAP) of the wheat and buckwheat flour and different wheat– buckwheat mixture before baking process

Flour	TP (mg GA/L extract)	FRAP (µmol Fe^{2+}/L extract)
WF	9.11 ± 0.14	55.45 ± 1.98
WF + WBF (85 % + 15 %)	9.24 ± 0.63	110.95 ± 3.57
WF + WBF (70 % + 30 %)	9.82 ± 0.77	208.95 ± 1.33
WF + WBF (60 % + 40 %)	10.84 ± 0.59	249.95 ± 4.92
WBF	17.91 ± 0.81	461.95 ± 2.87

*WF – wheat flour; WBF – wholegrain buckwheat flour

The samples of wheat flour with added buckwheat flour showed a considerably higher antioxidative activity compared to wheat flour alone (Table 3). With the increase of the share of buckwheat flour from 15% to 40%, antioxidative activity increased 2 to 5 times, while antioxidative activity in water extract of buckwheat flour (461.95 µmol Fe^{2+}/L extract) was 8 times higher than in wheat flour (55.45 µmol Fe^{2+}/L extract). This increase in antioxidative activity can be explained with the fact that the increase of buckwheat flour share leads to the increase in total phenols which are believed to have a considerable antioxidative activity.

The correlation coefficient ($r = 0.939$; $p \leq 0.05$) shows that there is a statistically significant correlation between the concentration of total phenols and antioxidative activity in water extract of tested flour samples. Table 4 shows the results of total phenols determination in wheat bread and in the samples of bread with added buckwheat flour. Similar to flour samples, the increase of buckwheat flour from 15% to 40% lead to the increase in total phenols concentration in bread samples (7.46 – 10.13 mg GA/L extract) compared to wheat bread (4.88 mg GA/L extract). Total phenol concentration was lower in all bread samples, regardless of the share of buckwheat flour, when compared to the samples of the flour used for breadmaking. Reduction of total phenol concentration can be explained by negative effects of baking process.

Table 4. Total phenols (TP) contents and antioxidant activity (FRAP) of the bread samples

Bread	TP (mg GA/L extract)	FRAP (µmol Fe^{2+}/L extract)
Wheat bread	4.88 ± 0.41	131.95 ± 3.01
Bread with 15% WBF	7.46 ± 0.36	231.95 ± 2.45
Bread with 30% WBF	9.19 ± 0.52	308.45 ± 4.88
Bread with 40% WBF	10.13 ± 0.39	368.45 ± 3.62

*WBF – wholegrain buckwheat flour

The highest loss of total phenol (46.43%) was observed between wheat bread and wheat flour. In bread samples containing 15%, 30% and 40% of buckwheat flour, the loss of total phenols compared to flour samples was 19.26%, 6.41% and 6.55%. Since phenol determinations have been done in water extract, the results can be interpreted as baking process having more significant effect on the loss of total phenols (water-soluble) in wheat flour than in buckwheat flour. Antioxidative activity of wheat bread was 131.95 µmol Fe^{2+}/L extract, while addition of buckwheat flour significantly increased antioxidativ activity. In bread samples with 15%, 30% and 40% of buckwheat flour, there was an increase of antioxidative activity by 43.11%, 57.22% and 64.19% compared to wheat bread. The correlation coefficients show that there is a statistically significant correlation between total phenols concentration and antioxidative activity in water extract of the tested bread samples ($r = 0.995$; $p \leq 0.05$). All bread samples showed higher antioxidative activity compared to flour samples. Although baking process lead to reduced total phenols concentration, the increase of antioxidative activity in bread samples can be explained by the formation of products of Maillard's reaction[20,21]. The biggest difference between antioxidative activity of bread and flour samples was observed

between wheat bread and wheat flour. The difference between antioxidative activity of samples of bread and flour with addition of buckwheat flour decreases with the increase of buckwheat flour share. Based on the information above, we can conclude that baking process had bigger influence on formation of products of Maillard's reaction in wheat bread than in breads with buckwheat flour. During storage total phenol content decreased in all samples. Antioxidative activity was reduced during storage (Table 5). The results of this analysis confirm the results published by other authors[22,23].

Table 5. Total phenols (TP) contents and antioxidant activity (FRAP) of the bread samples after storage (30 days storage at -22°C)

Bread	TP (mg GA/L extract)	FRAP (µmol Fe^{2+}/L extract)
Wheat bread	4.61 ± 0,73	121.95 ± 3.52
Bread with 15% WBF	7.15 ± 1,07	197.45 ± 1.19
Bread with 30% WBF	8.73 ± 0,55	262.95 ± 4.24
Bread with 40% WBF	9.54 ± 0,87	333.95 ± 4.08

* WBF – wholegrain buckwheat flour

Tables 6 and 7 shaws the results of determination of *L*, *a* and *b* values of the colour of the bread crumb and crust. The lowest levels of red (*a*) and yellow (*b*) pigment in the colour of the crumb have been measured in wheat bread. With the increase of WBF share in bread from 15% to 40% the level of yellow pigment (*b*) considerably increases, while *L* value considerably decreases compared to *L* value of wheat bread. Based on whiteness index (WI), the darkest bread crumb (WI=39.63) was found in the bread sample with 40% WBF, while wheat bread had the whitest crumb (WI=71.11). All bread samples had a higher level of red (*a*) and yellow (*b*) pigment in the crust than in the crumb. The crust of wheat bread had the highest level of yellow pigment (*b*).

Table 6. The values of whiteness index (WI) and parameters L, a and b for colour of bread crumb

Bread	*L*	*a*	*b*	Whiteness index (WI)
Wheat bread	74.62 ± 0.12^{a}	-1.63 ± 0.02^{a}	13.70 ± 0.10^{a}	71.11^{a}
Bread with 15% WBF	57.24 ± 0.11^{b}	1.20 ± 0.03^{b}	15.75 ± 0.09^{b}	53.83^{b}
Bread with 30% WBF	53.38 ± 0.39^{bc}	2.06 ± 0.08^{c}	17.38 ± 0.15^{c}	49.94^{c}
Bread with 40% WBF	41.79 ± 0.12^{c}	2.80 ± 0.05^{d}	18.13 ± 0.11^{d}	39.63^{d}

*WBF – wholegrain buckwheat flour

**Values in the same column marked with different letters are statistically significantly different (Duncan test; $P < 0,05$)

Table 7. The values of whiteness index (WI) and parameters L, a and b for colour of bread crust

Bread	*L*	*a*	*b*	Whiteness index (WI)
Wheat bread	69.54 ± 0.22^{a}	7.67 ± 0.16^{a}	33.45 ± 0.22^{a}	54.05^{a}
Bread with 15% WBF	55.37 ± 0.13^{b}	8.01 ± 0.03^{a}	30.16 ± 0.44^{b}	45.12^{b}
Bread with 30% WBF	48.72 ± 0.43^{c}	10.48 ± 0.24^{b}	28.37 ± 0.21^{c}	39.93^{c}
Bread with 40% WBF	45.86 ± 0.31^{d}	13.16 ± 0.33^{c}	22.69 ± 0.44^{d}	40.79^{c}

*WBF – wholegrain buckwheat flour

***Values in the same column marked with different letters are statistically significantly different (Duncan test; $P < 0,05$)

Table 6. The values of whiteness index (WI) and parameters L, a and b for colour of bread crumb

Bread	*L*	*a*	*b*	Whiteness index (WI)
Wheat bread	74.62 ± 0.12^{a}	-1.63 ± 0.02^{a}	13.70 ± 0.10^{a}	71.11^{a}
Bread with 15% WBF	57.24 ± 0.11^{b}	1.20 ± 0.03^{b}	15.75 ± 0.09^{b}	53.83^{b}
Bread with 30% WBF	53.38 ± 0.39^{bc}	2.06 ± 0.08^{c}	17.38 ± 0.15^{c}	49.94^{c}
Bread with 40% WBF	41.79 ± 0.12^{c}	2.80 ± 0.05^{d}	18.13 ± 0.11^{d}	39.63^{d}

*WBF – wholegrain buckwheat flour
**Values in the same column marked with different letters are statistically significantly different (Duncan test; P < 0,05)

Table 7. The values of whiteness index (WI) and parameters L, a and b for colour of bread crust

Bread	*L*	*a*	*b*	Whiteness index (WI)
Wheat bread	69.54 ± 0.22^{a}	7.67 ± 0.16^{a}	33.45 ± 0.22^{a}	54.05^{a}
Bread with 15% WBF	55.37 ± 0.13^{b}	8.01 ± 0.03^{a}	30.16 ± 0.44^{b}	45.12^{b}
Bread with 30% WBF	48.72 ± 0.43^{c}	10.48 ± 0.24^{b}	28.37 ± 0.21^{c}	39.93^{c}
Bread with 40% WBF	45.86 ± 0.31^{d}	13.16 ± 0.33^{c}	22.69 ± 0.44^{d}	40.79^{c}

*WBF – wholegrain buckwheat flour
***Values in the same column marked with different letters are statistically significantly different (Duncan test; P < 0,05)

The lowest brightness of the crust was measured in samples with 30% (WI=39.93) and 40% (WI=40.79) buckwheat flour. The increase of WBF share in bread from 15% to 40% significantly increases the level of red pigment (*a*) in bread crust colour.
The results of the statistic analysis (Table 8) show that there is a correlation between certain parameters of colour (*L*, *a* and *b*) and total phenol concentration and antioxidant activity. The correlation was especially significant between *b* value for the colour of bread crumb (yellow pigment level) and total phenols ($r = 0.999$; $p \leq 0.01$) and FRAP ($r = 0.996$; $p \leq 0.01$)

Table 8. The coefficients of correlation between total phenols (TP), FRAP and L, a and b colour values for the crumb and crust of bread

	WI*** crumb	WI crust	*L* crumb	*a* crumb	*b* crumb	*L* crust	*a* crust	*b* crust
TP	-0.98*	-0.969*	-0.977*	0.987*	0.999**	-0.993**	0.884	-0.924
FRAP	-0.982*	-0.941	-0.981*	0.973*	0.996**	-0.979*	0.922	-0.954*

*Correlation is significant at the 0.05 level.
**Correlation is significant at the 0.01 level.
***WI - whiteness index

CONCLUSION

Wholegrain buckwheat flour is a good source of phenols and possessed good antioxidant activity. Replacement of 15% to 40% wheat flour in bread formulation with wholegrain buckwheat flour can increase total phenol concentration and improve antioxidant activity of the bread. Baking process influenced more significantly the loss of total phenols in wheat flour than in buckwheat flour. Addition of buckwheat flour causes a darker colour of the bread crumb and crust.

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STUDYING OF CORROSION BEHAVIOUR OF 316L STEEL AS A METALLIC BIOMATERIAL IN THE INFUSION SOLUTION

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

In this paper, corrosion behaviour of alloying metals and stainless steel 316L in the infusion solution is given. Voltammogram for pure metals (Fe, Cr, Ni, Mn, Mo) and a metal implant in infusion solution at 37ºC has been recorded, indicating that the presence of pure metals in steel 316L, besides contributing to the mechanical properties, certainly their great contribution is in the total corrosion stability of metallic implants.
Given that, the biocompatible materials are used, not only in orthopaedics, but in other fields of medicine, where these interact with living tissues, it can be concluded that for the development of new materials for applications in medicine, very significant knowledge and understanding of the above mentioned interaction and because of that biocompatibility and nontoxicity materials become very important and critical factors for further development of metallic implant materials.
Cyclic voltammograms recorded in the infusion solution clearly indicate the occurrence of pitting corrosion.

Key words: stainless steel 316L, infusion solution, pitting corrosion.

INTRODUCTION

Metallic biomaterials are strictly defined materials used in contact with cells, tissues or body fluids of living organisms.
Stainless steels, cobalt-chromium alloys and titanium alloys are basic metallic and biocompatible biomaterials in biomedical engineering.
Steel materials have already begun to be used for making plates and screws for fixing broken bones during the twentieth century.
Because of the corrosion occurring in the human body on these implants, primarily used steels alloyed with vanadium and coated by nickel were replaced by carbon steels.
However, these steels were not sufficiently resistant to corrosion, which resulted in their negative influence on the human body.
Research in the field of biocompatibility of metallic materials have contributed to the development of stainless steel, cobalt-chromium-nickel-molybdenum alloys and titanium alloys, which have gradually become the main biocompatible medical materials for use in orthopaedics and in other fields of medicine.
Numerous biomaterials and medical devices today are normally used as a means of dental prosthetic, orthopaedic, cardiovascular, ophthalmologic and reconstructive surgery. They are successfully used in interventions, such as angioplasty (stents) and haemodialysis (membranes), for surgical stitches or bioadhesive, but as devices for controlled release of drugs. Most implants well serve their carriers for a specified period for the

purpose for which they were intended. However, some implants and extracorporeal devices inevitably create complications, whether as a result of inflammation, infection, interaction in the form of unwanted allergic or toxic reactions, or due to demanding devices, which can cause a variety of harmful consequences, and even death of the host. Complications are usually the result of biomaterial-tissue interactions that occur at the site of installation of each material, although they may have a systemic or general character. Effects of implants on the host tissue and the living tissue to the implant are equally important and to avoid possible complications and to prevent malfunctions or cancelling devices. For the application of biomaterials is important biocompatibility with tissue, mechanical continuity with the surrounding bone tissue, nontoxicity biomaterials or their degradation products, as well as price. Broad set of biomaterials differ from each other in the chemical, physical and mechanical terms. Thereby, the application included many anatomical sites in the human body. The mechanisms through which the body reacts to foreign bodies and heals injuries are observed in each specific case. Problems, care about them, or unexplained observations are common in implants.

The first metal alloy developed specifically for human use was vanadium steel, which was used to manufacture bone fracture plates (Sherman plates) and screws - but then metal alloy experienced a wider use in making medical and dental implants.

Metals and metal alloys have good characteristics of which we should emphasize titanium and its alloys because of its good resistance to corrosion, biocompatibility and less stiffness that allows good transmission of mechanical stress of this implant to the bone.

TiO_2 on the surface has bioactive properties and induces the growth of new bone. Ti Ni - alloys with shape memory effect are also interesting for applications in orthopaedics. Co Cr – alloys as Ti are passive in the human body, and are used a lot in orthopaedics.

The first stainless steel wider used for implant fabrication was the 18-8 (type 302 in newer classification), which is stronger and more resistant to corrosion than the vanadium steel as a firstly used metallic biomaterials, and then abandoned due to inadequate corrosion resistance in vivo. Later, the 18-8 Mo stainless steel (known as 316) was introduced, which contains a small percentage of molybdenum to improve the corrosion resistance in the salt water. Furthermore, the carbon content of the 316 stainless steel was reduced from 0.08 wt% to a maximum of 0.03 wt% in the so-called type 316L stainless steel, for better corrosion resistance to chloride solutions.

The minimum concentration of chromium in stainless steels is 12 wt%, although the chromium is a reactive element, it and its alloys can be passivated giving exceptionally rigid steel to corrosion.

Corrosion can be defined as unwanted chemical or electrochemical reaction of metal with the environment, resulting in the continuous degradation to the oxides, hydroxides or other compounds.

Corrosion resistance is a characteristic of resistance of material to action of the surrounding medium.

The material in the same external conditions leads to less damage on the surface or to undesirable changes in microstructure is corrosion steadier. The basic characteristic of metal corrosion is

that it starts at the metal surface, where it quickly or slowly expanding in depth, and leads to changes in the composition of metals and their properties.

Basic thermodynamic relations between primary and secondary electrochemical reactions and secondary chemical reactions during corrosion of metal in an aqueous medium give E-pH diagrams introduced by Marcel Pourbaix.

These diagrams include the regions of immunity, passivity and corrosion.

Metal in the immunity cannot corrode, in the passivity can be (but must not) be passivated, and in the corrosion must corrode. Speed and flow of corrosion cannot be predicted on the basis of these diagrams.

Thus, these diagrams show the conditions in which the metal is thermodynamically stable, or can react, forming ions or complex compounds. Diagrams contain only thermodynamic data and there is no information about corrosion rate on them, but they have a large application in considering the problem of corrosion and therefore they allow prediction of the direction of spontaneous reaction, evaluating the composition of corrosion products and the ability to change the external environment in order to prevent or reduce its corrosion effect.

The significance of the Pourbaix diagram is that different parts of the body have different pH values. Consequently, a metal which performs well (immune or passive) in one part of the body may suffer an unacceptable amount of corrosion in another part. Moreover, pH can change dramatically in tissue that has been injured or infected. In particular, normal tissue fluid has a pH of about 7.4, but in a wound it can be as low as 3.5, and in an infected wound the pH can increase to 9.0.

Figure 1 shows Pourbaix diagram for chromium, showing regions associated with the effects of various body fluids [1,2,3,4].

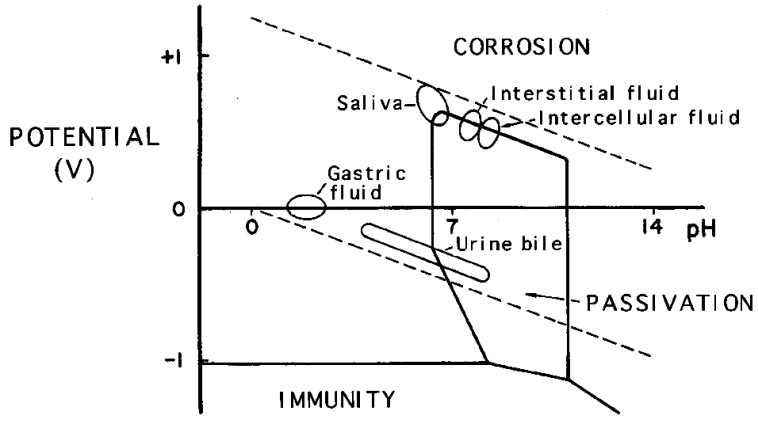

Figure 1. Purbaix diagram for Cr, showing regions associated with the effects of various body fluids

MATERIALS AND METHODS

As a testing material pure metals and stainless steel have been used:

- ✓ Iron, Spectrographically pure iron, Johnson Matthey Chemicals Limited Hatton garden London, EC.I England

- ✓ Nickel, Nickel rods, Specpure, Johnson Matthey Chemicals Limited Hatton garden London, EC.I England
- ✓ Molybdenum, Molybdenum rods, Spectrographically standardised substances, Matthey Hatton garden London, EC.I
- ✓ Manganese, Spectrographically pure
- ✓ AISI 316L stainless steel.

Table 1. The chemical composition of an AISI 316L stainless steel

Composition in weight percentages							
Cr	Ni	Mo	Mn	C	Si	P	Fe
17-19,5	13-15,5	2,5-4	2	0,03	0,75	0,03	to 100

Chromium is a metal of extraordinary mechanical and chemical properties. It has a good resistance to corrosion in many aggressive environments, and it is used in the production of alloy highly resistant to corrosion (Cr-Ni stainless steels) that has good quality. Standard electrode potential of chromium is -0.744 V. Although chromium has a negative standard redox potential it is resistant to corrosion due to the passive state which easily occurs on its surface.

Nickel is also technically important metal because of its good physical, mechanical and technological properties and corrosion resistance. It is resistant to atmospheric agents, resistant to sea water, cold non-oxidizing acids, alkaline solutions and molten strong bases. Nickel alloys are known for its resistance to corrosion and resistance to high temperatures, so they belong to the so-called "superalloys".

A small percentage of molybdenum in steel provides high hardness, which is maintained even at high temperatures. It is highly resistant to corrosion, practically insoluble in alkali solutions. It shows much greater resistance to corrosion in chloric medium, what present the human body and therefore it is used for making metal implants and surgical equipment. Manganese is largely applied in metallurgy, the addition of small amounts of manganese to molten steel, manganese acts as a powerful antioxidant removing oxygen and sulphur. It increase the hardness of steel and abrasion resistance when is added at higher levels.

Under normal conditions, the body fluids of the human body are 0.9% saline solutions containing amino acids and proteins. Body fluids consist of various fluids, such as tissue fluid, lymph and blood, but also contain solid components, such as the traveling cells (leukocytes) and blood particles (lymphocytes, platelets and erythrocytes).

Empirical evidence suggests that corrosion of metals is due to the presence of Cl-ions, and from that reason the infusion solution containing 0.9% NaCl has been used for the monitoring and simulation of corrosion of metal implants in living organisms.

It was investigated corrosion of the metallic implant steel 316 L and pure metals (Fe, Cr, Ni, Mn, Mo) in the infusion solution by voltammetric linear polarization, cyclic voltammetry and Tafel extrapolation. Voltammetry involves electrochemical methods in which the system under

examination is applied electric voltage as an excitation signal where occurs a polarization of the working electrode, and as a response of the system registers the current cell.

Electrochemical measurements were carried out in three-electrode electrochemical cell. Working electrode was made out of nickel, chromium, manganese, iron and molybdenum spectroscopic purity, and the implant was made out of 316L stainless steel. Reference electrode was Ag/AgCl (E = 0.222V). The electrodes are connected to an electronic device (potentiostat/ galvanostat PAR EG&G model 263A), and by this one is controlled an electric voltage by electrochemical software with computerized management.[5,6].

RESULTS AND DISCUSSION

Corrosion behaviour of alloying metals and stainless steel in the infusion solution at body temperature of the human body (37 °C) was investigated, Figure 2. Corrosion potential values by extrapolation of the line where occurs a linear dependence of the current intensity vs. potential in the part where we have an electrochemical oxidation reaction of samples. In Table 2 potentials are given.

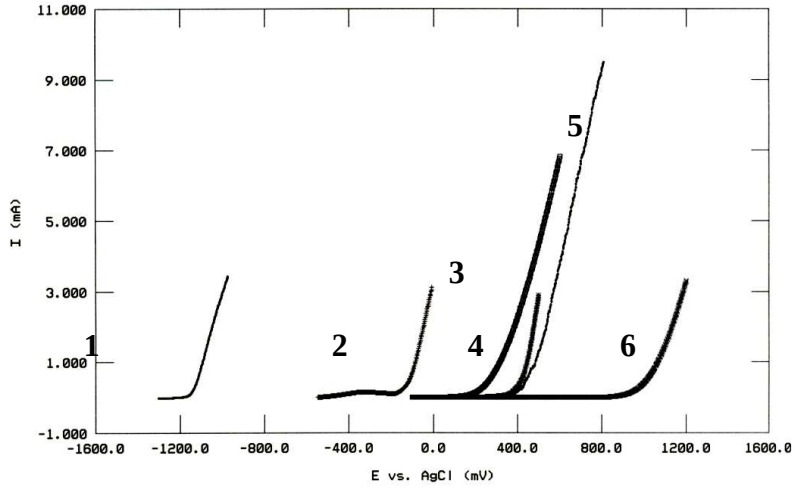

Figure 2. Summary voltammograms for pure metals and metal implant in the infusion solution at 37 °C; 1-Mn, 2-Fe, 3-Mo, 4-Ni, 5-metal implant, 6-Cr

Table 2 Oxidation potentials of samples in the infusion solution

Sample	Corrosion potential, V
Manganese	-1,140
Iron	-0,120
Molybdenum	0,260
Nickel	0,410
Implant	0,500
Chromium	0,990

Diagram 2 and Table 2 show that the oxidation potential is least therefore has a negative value for manganese, then raises for iron over molybdenum and nickel to chromium. Metal implant has oxidation potential values within the values for the pure metals, i.e. has more positive value of all metals that enter into the composition of the implants except chromium.

Therefore, beside of mechanical properties that metals give to the implant, certainly

their great contribution is to the overall stability of the metal implants.

Cyclic anodic polarization was performed in order to determine the characteristic parameters such as pitting potential and protection potential (repassivation potential) from polarization curves, Figure 3.

Local corrosion caused by anodic polarization can be stopped by repassivation during the back polarization process. Such an occurrence repassivation follows reduction in electricity and in a cyclic polarization curves appear hysteresis loop[7].

Cyclic voltammogram recorded in the infusion solution at two different temperatures, shows that the implant is more stable at 25 °C (curve 1) because the pitting potential is shifted to more anodic area compared with the curve 2 recorded at 37°C[6]. Scan rate used for linear polarization and cyclic voltammetry was 5 mV/s.

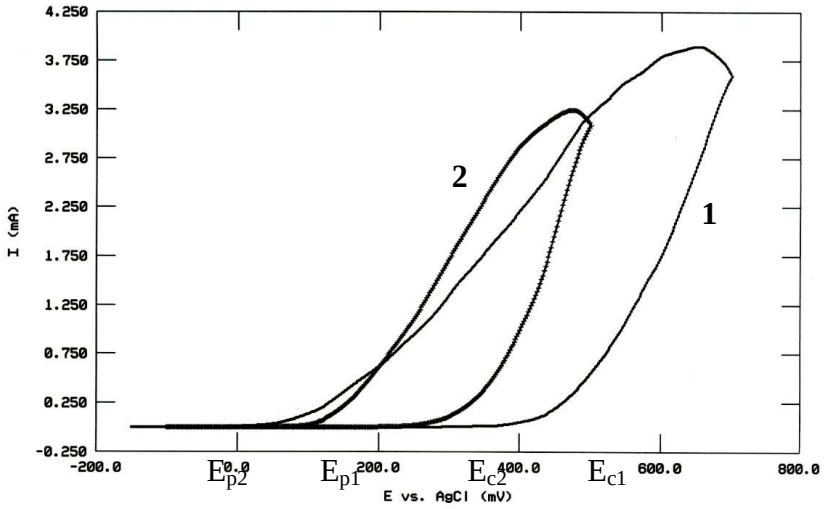

Figure 3. Cyclic voltammogram for metal imlant in infusion solution; (1) t = 25 °C; (2) t = 37 °C, v = 5 mV/s

CONCLUSION

Summary voltammogram for pure metals and metal implant recorded in the infusion solution at 37 °C, indicate that the presence of pure metals in steel 316L, besides contributing to the mechanical properties, certainly their great contribution is to the overall corrosion stability of the metal implant. Namely, the values of the oxidation potential of the implant are within the values for the pure metals. Metal implant tends local corrosion i.e. pitting corrosion. Cyclic voltammograms recorded in the infusion solution clearly indicate the occurrence of pitting potential, protective potential and corrosion current. Pitting potential is less positive in infusion solution at a temperature of 37 °C. Since biocompatible metal materials are widely used in medicine, while being in constant interaction with living tissues, it can be concluded that the development of new materials is extremely significant knowledge and understanding of the mentioned interactions, and from that reason biocompatibility, corrosion resistance and nontoxicity becoming critical factors for further development of metallic implant materials.

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INFLUENCE OF MULCHING AND DIRECT PLANT COVERING ON THE NITRATE CONTENT IN LETTUCE

REVIEW ARTICLE

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ABSTRACT

In the technology of winter salads, mineral nutrition, fertilization, genotype and production method are very important for achieving high yield and quality salads. Based on this set a goal to work to determine how the different genotype and production methods affect nitrate accumulation in lettuce.

The experiment was conducted in the winter cycle of growing lettuce 2009th and 2010th The varieties using Archimedes RZ, Santoro RZ and Kibou RZ grown on uncovered soil, under black PE foil, under the agrotextile and combination of black PE foil + agrotextile

The experiment was conducted by a random bloc system repeating it four times in experimental the field of Faculty of Agriculture East Sarajevo.

A two-year study showed a tendency of nitrate increase with various means of production. The minimal level of nitrate was in all three lettuce cultivars on control variante (Archimedes 3020,12 mg/kg; Santoro 2145,75 mg/kg and Kibou 2026,75 mg/kg), however the maximal level of nitrate was in the variant of agrotekstile (Archimedes 3266,10 mg/kg; Santoro 2888,50 mg/kg and Kibou 2423 mg/kg).

Key words: lettuce, nitrate, genotype, soil mulching.

INTRODUCTION

Lettuce is particularly important in the diet because of the high content of scarce micro-nutritious elements such as copper, zinc, iron and magnesium, and even during the period (spring and autumn) when there is the greatest demand of them (A. Miskovic, et al 2001; Lazic Branka 2001).

The biochemical composition of the edible part of vegetables depends on genotype and agro technique applied (Djurovka et al., 1988; B. Lazic, et al., 2000). According to the researches by the aforesaid authors, the aim of use of these new technologies in vegetable production is profitability, with the rational use of all inputs oriented towards the quality and environmental protection. There are also significant results (Skoric M., 1996; Bosnjak D. 2005) which indicate that there is increase in application of dripping irrigation with covering of the soil by adequate foil and application of nutrient elements, all in order to create optimal conditions for growth and development throughout the whole vegetation period**,** which makes a quality market product.

Based on current affairs issues of health and the safe production of vegetables set a goal to work to determine how and to what extent the different genotype and production technologies affect nitrate accumulation in lettuce.

MATERIALS AND METHODS

The tests were carried out during a two-year period (2009-2010) on three genotypes in a greenhouse without additional heating, on the experimental field of the Faculty of Agriculture in East Sarajevo.The experiment was conducted by the random bloc system repeating it four times on a 2.4 m^2 (0.3 x 8 m) experimental parcel with three beds, one bed for each cultivar.

Nursery plants were grown in containers with 40 openings (10 x 4) of dimension h =4,0cm, vol=70cc, in the greenhouse without additional heating. They were sown at the beginning of September using coated seed. The containers were filled with Klasmann substrate.

The 25 days old nursery plants were transplanted in the beds at 20 cm distance between plants, and 30 cm between the beds creating an area of nearly150 000 plants/ha.As for irrigation, we used a system "drop by drop" which was placed together with mulch.

We tested two factors: mulching (control, covering of black PE foil, agrotextile, combination of mulch + agro textile and genotype (Archimedes RZ,Santoro RZ,Kibou RZ) .

Picking of lettuce was conducted at the harvest maturity. Nitrates are determined by a photometrical method (Marjanović, 1998).

The results achieved were processed by variance analysis method of a two-factorial trial (ANOVA) using SPSS 4.5 software.We carried out the testing of significance of differences between the means by the method of the variance analysis of two-factorial trial covering x variety (4 x 3). The significance of differences of individual means was tested by LSD test for the general means and interaction.

RESULTS AND DISCUSSION

Temperatures in the greenhouse were directly dependent on T in the open field after the greenhouse is not warmed up. During hot days the temperature difference was 7.64^{0}C, while during the cold days had values between 2.98- 3.82^{0}C. During the growing season salads temperatures were within the optimal values (chart 1.) there were no significant fluctuations.

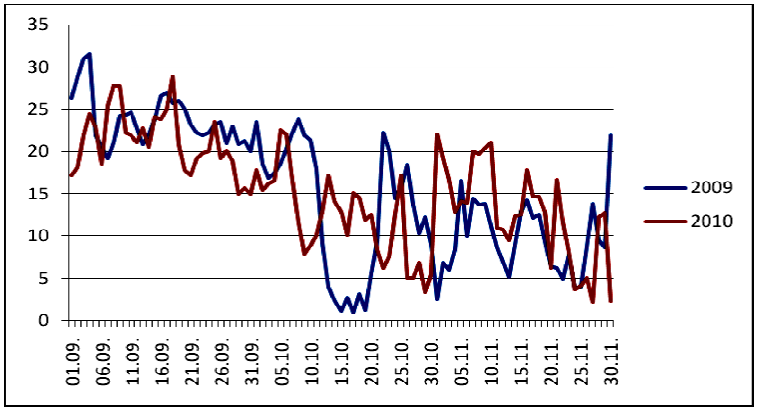

Chart 1. Daily flow T in the greenhouse during the experiment

Nitrate content in lettuce depends on many factors. There is more nitrate in vegetables cultivated with higher doses of nitrogen and organic fertilizers at low relative air humidity, under drought conditions, low light intensity, during the short day, temperatures above 25 C degrees (Kastori et al., 1998).

According to the researches of Poulsen et al., (1995) the nitrate content decreases from the outer leaf fractions towards the inner ones (1850-650 mg/kg) depending on the level of nitrogen added (455-230 mg/kg). According to Kastori (1998), there is more nitrate in the peduncle of the leaf than in the leaf.

Research results (Table 2) indicate that all four types of covering were different from one another in nitrate content. The highest content of nitrate (3192.25 mg/kg) was recorded in agro textile and the lowest one (2597.83 mg / kg) on the control.

Depending on the choice of genotypes, nitrate content ranged from 2606.12 mg / kg to 3464.56 mg/kg.

Table 1. Mean values of nitrate (mg/kg) in lettuce in 2009

Covering land	Genotypes			Average
	Archimed	Santoro	Kibou	
Control	3313.00	2290.00	2190.50	2597.83
Covering of black PE foil	3491.50	2403.00	2523.00	2805.83
Agrotekstile	3642.50	3253.00	2681.00	3192.25
combin. of mulch + agro textile	3411.25	2782.25	3029.75	3074.41
Average	3464.56	2682.06	2606.12	2917.58

LSD	A	B	AxB
0.05	97.04	84.03	168.08
0.01	129.70	112.32	224.66

In the second year of researches, the average value of nitrate content (Table 2) ranged from 2197.25 mg/kg (control) to 2526.25 mg/kg (agro textile).

The differences gained in the average values of nitrate content at different genotypes of covering are rated at the threshold of significance of 1%, only the differences stated between the third and fourth variant of covering were not statistically justified. In the studies on soil mulching with black film, Benoit and Ceustermans (1992) demonstrated a significantly higher nitrate content in lettuce grown on mulched soil as compared to non-mulched cultivation.

Table 2. Mean values of nitrate (mg/kg) in lettuce in 2010

Covering land	Genotypes			Average
	Archimed	Santoro	Kibou	
Control	2727.25	2001.5	1863.00	2197.25
covering of black PE foil	2831.75	2211.00	2136.50	2393.08
Agrotekstile	2889.70	2524.00	2165.00	2526.25
combin. of mulch + agro textile	2923.00	2148.50	2487.00	2519.50
Average	2842.90	2221.25	2162.87	2409.02

LSD	A	B	AxB
0.05	61.61	53.34	213.47
0.01	82.35	71.30	285.33

In the second year of researches, the order of the varieties studied, in terms of this trait, was identical to the one in the previous year. Archimedes genotype had the highest average value of nitrate content (2842.9 mg/kg), and Kibous genotype had the lowest one (2162.87 mg/kg). The ability to accumulate nitrate is genetically controlled and is also influenced by the nitrate content of the soil, climate, fertiliser and genotype (Abu-Rayyan et al., 2004). Kosović's researches (1989), too, have shown that nitrate accumulation depends on the type, genotype and climatic conditions.

Similar results are found in the works of Lazic et al., (1990, 1994, 2002). According to their researches, the nitrate content is a varietal characteristics and leafy lettuce has the highest level of it (350.30 mg/kg fresh weight), and roman salad the lowest (310, 90 mg/kg fresh mass).

CONCLUSION

Based on a work goal and two-year experiment can be carried out the following conclusions:

- The nitrate content estimated as a harmful substance for human consumption depended on the method of production. There is an emphasized trend of increasing nitrate with the application of various methods of production. The highest nitrate content (5718.50 mg/kg) was in the variant of covering soil with agro textile, which is by 16% more compared to the control variant (4795.08 mg/kg).
- Genotypes differ significantly in nitrate content. The highest content was the genotype Archimedes 3153 mg/kg, while the lowest content of the genotype Kibou 2384,49 mg/kg. The European Union establishes the maximum permissible levels from 3500 to 4500 mg/kg fresh weight for the winter season and 2500 mg/kg for the summer crops (Europe, 2009).

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