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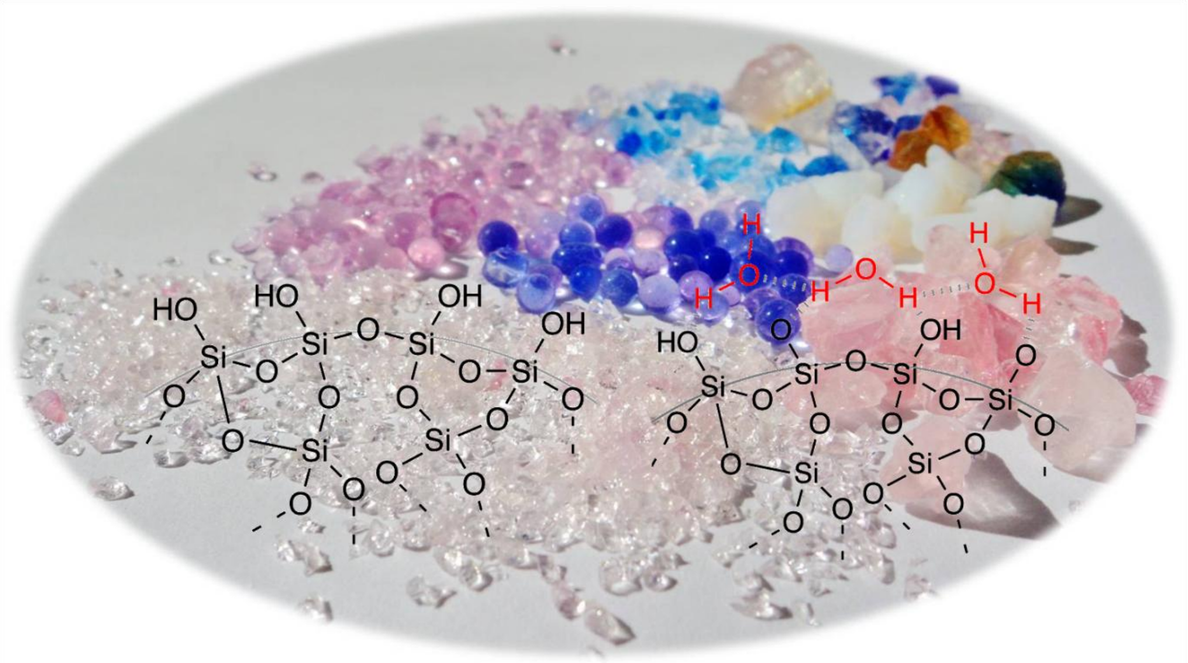

42

Juni 2014.

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Faculty of Science Sarajevo

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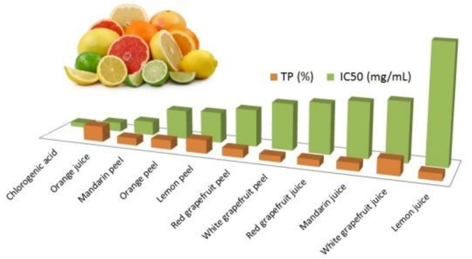

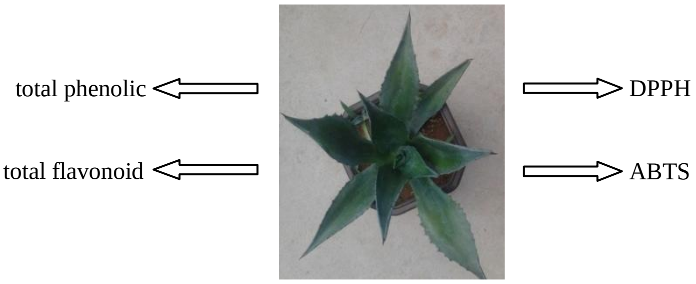

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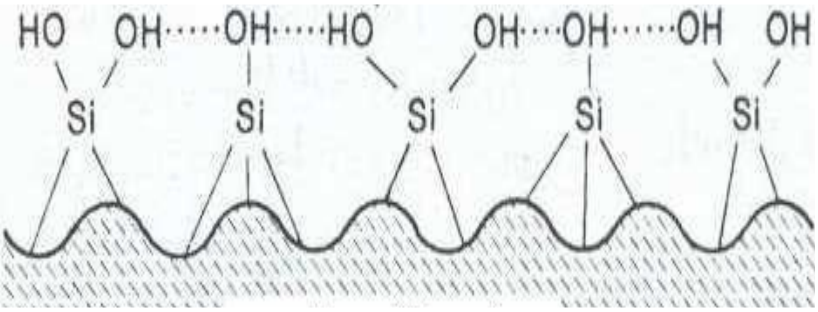

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Said Delić
Nevzet Merdić

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	Volumetric method	XRF	Volumetric method	XRF
	Content %		Content %	
SiO_2	27,4	27,6	13,8	13,7
Al_2O_3	8,45	8,73	3,18	2,31
Fe_2O_3	3,86	3,25	1,91	1,84
CaO	29,2	29,8	43,6	44,5
MgO	1,64	1,53	0,80	0,54
K_2O	-	0,99	-	0,29

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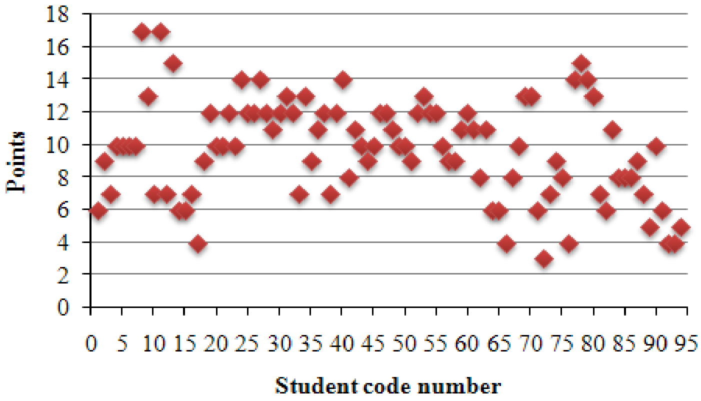
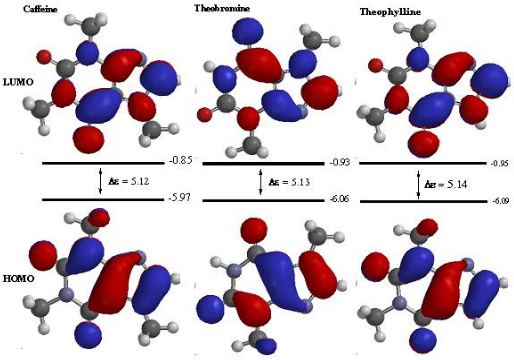
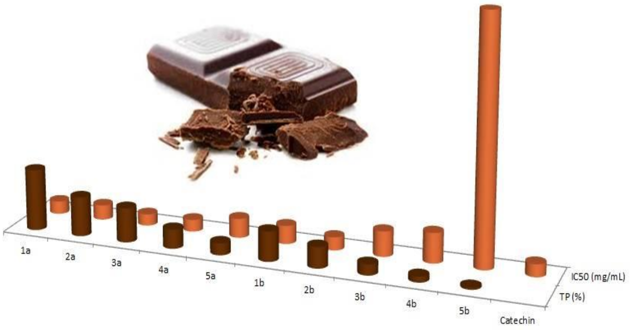
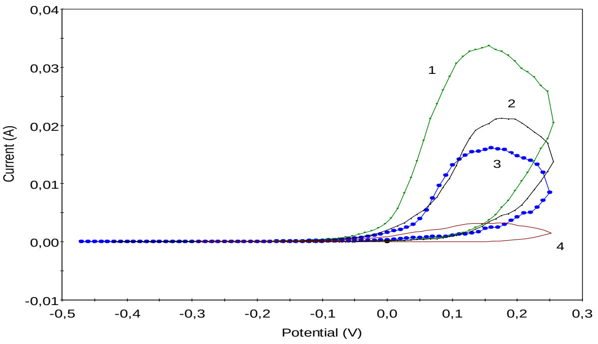
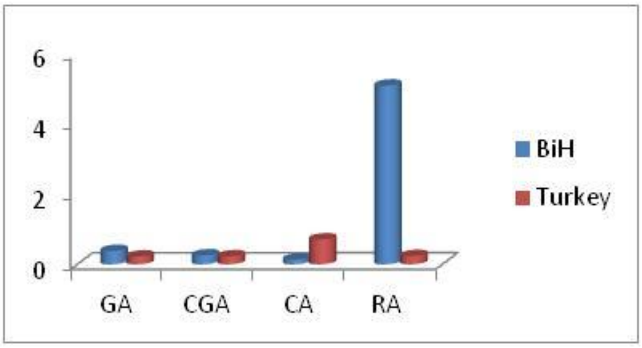
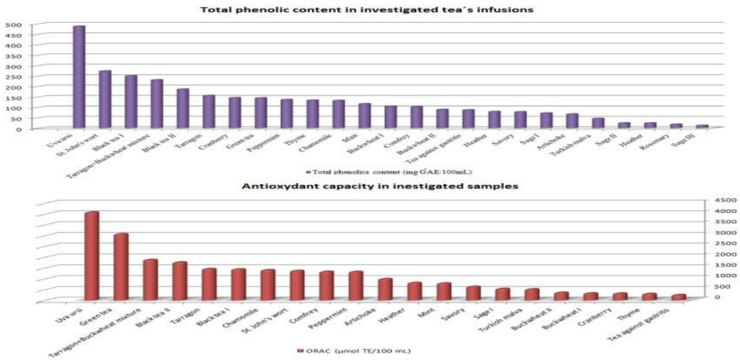



Caffeine
Theobromine
Theophylline
LUMO
-0.85
ΔE = 5.12
-5.97
-0.93
ΔE = 5.13
-6.06
-0.95
ΔE = 5.14
-6.09
HOMO


1a
2a
3a
4a
5a
1b
2b
3b
4b
5b
Catechin
IC50 (mg/mL)
TP (%)


0,04
0,03
0,02
0,01
0,00
-0,01
Current (A)
-0,5
-0,4
-0,3
-0,2
-0,1
0,0
0,1
0,2
0,3
Potential (V)
1
2
3
4


6
4
2
0
GA
CGA
CA
RA
BiH
Turkey


Total phenolic content in investigated tea´s infusions
500
450
400
350
300
250
200
150
100
50
0
Uva ursi
St. John's wort
Black tea I
Tarragon+Buckwheat mixture
Black tea II
Tarragon
Cranberry
Green tea
Peppermint
Thyme
Chamomile
Mint
Buckwheat I
Comfrey
Buckwheat II
Tea against gastritis
Heather
Savory
Sage I
Artichoke
Turkish malva
Sage II
Heather
Rosemary
Sage III
Total phenolics content (mg GAE/100mL)
Antioxydant capacity in inestigated samples
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Uva ursi
Green tea
Tarragon+Buckwheat mixture
Black tea II
Tarragon
Black tea I
Chamomile
St. John's wort
Comfrey
Peppermint
Artichoke
Heather
Mint
Savory
Sage I
Turkish malva
Buckwheat II
Buckwheat I
Cranberry
Thyme
Tea against gastritis
ORAC (µmol TE/100 mL)

Editorial

Society of Chemists and Chemical Engineers of Canton Sarajevo was founded in the year 2000 with an idea to promote chemistry in different sectors of our community. Its aim is to develop and improve the scientific, professional and educational activities in all areas of pure and applied chemistry. In Sarajevo, for the first time ever, Congress of Chemists and Chemical Engineers of Bosnia and Herzegovina with international participation will be held from 10th to 12th October, 2014. The conference is organized by the Society of chemists and technologists of the Sarajevo Canton and Faculty of Natural Science, Sarajevo. This conference will initiate and organize meetings of scientists doing research in different fields of chemistry and chemical technology, which will, I sincerely hope, result in a more intensive scientific cooperation, exchange of scientific knowledge and professional experience, and potential international scientific projects in perspective.
The scientific program will be of high quality with a great number of interesting topics and educational lectures with subjects as:

- Analytical and Environmental Chemistry
- Biochemistry and Biotechnology
- Inorganic Chemistry
- Biological Chemistry
- Organic and Medicinal Chemistry
- Physical and Theoretical Chemistry
- Chemistry of Advanced Materials
- Chemical Engineering
- Education in Chemistry
- Topics related to Chemistry

Main speakers from four different university centers of Europe will take a part in Congress and present their research giving it truly an international character.

Selected papers presented at this meeting will be published in the Bulletin of Chemists and technologists of BiH, journal that has been publishing research papers since 1952 year.

Editors

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Original scientific article

Phenolic Compounds and Antioxidant Activity of Some Citruses

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Abstract: Citrus fruits (Rutaceae) are important source of phenolic compounds and their glycosides. These compounds are phenolic acids for bioactive responsible for the antioxidant and many other biological activities. In this paper five extracts from juice and peel of different types of citruses (tangerine, lemon, pink grapefruit, white grapefruit, and orange) were analyzed for total phenolic content and antioxidant activity. Total phenolic content was determined by spectrophotometric Folin-Ciocalteu method, and the values varied from 0.192 ± 0.015 mg GAE/mL for white grapefruit peel to 0747 ± 0098 mg GAE/mL for white grapefruit juice. Antioxidant activity of samples was tested using the total antioxidant method that implies reduction of molybdenum ions and it is expressed as IC_{50}. The IC_{50} values were ranged from 6.00 ± 0.50 mg/mL for orange juice to 78.11 ± 6.70 mg/mL sample of lemon juice.

INTRODUCTION

Phenolic compounds, as secondary metabolites, are widely distributed in plants. Plant phenolics are generally involved in defense against ultraviolet radiation or aggression by pathogens, parasites, as well as contributing to plants' colors. They are widespread constituents of plant foods (fruits, vegetables, cereals, olive, legumes, chocolate, etc.) and beverages (tea, coffee, beer, wine, etc.), and partially responsible for the overall organoleptic properties of plant foods (Shahidi & Naczak, 2004).

Despite their wide distribution, the health effects of dietary polyphenols have come to the attention of nutritionists only in recent years. Researchers and food manufacturers have become more interested in polyphenols due to their potent antioxidant properties, their abundance in the diet, and their credible effects in the prevention of various oxidative stress associated diseases (Gornistein *et al*., 2001; Manach *et al*., 2004; Li *et al*., 2006). The preventive effects of these second plant metabolites in terms of cardiovascular, neurodegenerative diseases and cancer are deduced from epidemiologic data as well as *in vitro* and *in vivo* (Hertog *et al.*, 1994; Cole *et al.*, 2005) and result in respective nutritional recommendations.

Phenolic compounds are excellent antioxidants due to their ability to donate an electron or hydrogen from phenolic hydroxyl groups. Resulting phenoxy radical tends to be poorly reactive because of electron delocalization in the aromatic ring, and therefore reactive radical is replaced by a radical of limited activity (Li *et al*., 2006; Shahidi and Naczak 2004; Topčagić, 2009).

Citrus is the general term for plants belonging to the family Rutaceae. Plants have large shrubs or small trees that can reach a height of 5-15 m with branches covered with thorns and evergreen leaves (Ladaniya, 2008). They are important source of many bioactive compounds, such as phenolic acids and flavanone glycosides. Naringin and hesperidin, so-called citrus flavonoids, are the two main glycosidic flavanones presented in citruses (Abeysinghe *et al*., 2007). Caffeic, chlorogenic, ferulic, sinapic and *p*-coumaric acid are the most abundant phenolic acids present in citruses (Tokusoglu & Hall, 2011).

The aim of this study was to determine the content of phenolic compounds and total antioxidant activity in extracts isolated from several species of citruses (tangerine, orange, red grapefruit, white grapefruit, and lemon).

EXPERIMENTAL

Isolation

Mandarin, orange, white grapefruit, pink grapefruit, and lemon, were purchased at a local supermarket in October 2012.The peel is separated from the edible part, dried at room temperature and grinded. Then, 1 g of each sample was extracted with ethanol using ultrasonic bath. All samples were filtered and stored in a refrigerator at 4°C in glass bottles, until analysis.

Edible part was squashed and the juice was filtered through gauze and stored in a refrigerator at 4°C in plastic bottles, until analysis.

Determination of total phenolics

Total phenolic content was measured using Folin-Ciocalteu spectrophotometric method (Singleton & Rossi, 1965), using gallic acid for calibration curve. All tests were performed in triplicates, and results are presented as gallic acid equivalents.

Antioxidant activity

Antioxidant activity of isolated extracts was tested using total antioxidant capacity spectrophotometric method (Prieto *et al*., 1999). Method is based on the ability of potent antioxidant to reduce molybdenum ions. All tests were performed in triplicates, and results are presented as IC_{50} values that indicate the concentration of extracts that reduces the 50% of molybdenum. Catechin was as standard probe.

RESULTS AND DISCUSSION

Isolation of phenolic compounds from peel and juice of citrus fruits was performed using ultrasonic extraction. The yields isolated from the peel were in the range from 4.91% to 7.44%, for orange and white grapefruit, respectively.

Determination of the total content of phenolic compounds was performed using spectrophotometric Folin-Cicolateu method.

Results from spectrophotometric determination of total phenolic content in isolated extracts are summarized in Table 1 as mg of gallic acid equivalent per gram of extract, and as content of phenolic compounds in extract. Values are represented as the mean taking into account the standard deviation. The total content of phenolic compounds of peels of citrus fruits ranging from 0.192 ± 0.015 mg GAE/mL for white grapefruit to 0.480 ± 0.007 mg GAE/mL for lemon, while total phenolics for juices were ranged from0.322 ± 0.002 mg GAE/mL for lemon to 0.747 ± 0.098 mg GAE/mL for white grapefruit.

Based on the results, it can be concluded that the content of phenolic compounds is generally higher in juice samples.

Table 1: The phenolic content of extracts of citrus fruits.

Sample	**Total phenolic content** **mg GA/mL**	**%**
Lemon peel	0.480 ± 0.007	0.89 ± 0.01
Lemon juice	0.322 ± 0.002	0.46 ± 0.04
Orange peel	0.452 ± 0.027	0.61 ± 0.04
Orange juice	0.437 ± 0.002	1.02 ± 0.04
Mandarin peel	0.334 ± 0.014	0.49 ± 0.02
Mandarin juice	0.357 ± 0.033	0.51 ± 0.05
Red grapefruit peel	0.283 ± 0.018	0.55 ± 0.03
Red grapefruit juice	0.359 ± 0.029	0.44 ± 0.04
White grapefruit peel	0.192 ± 0.015	0.39 ± 0.03
White grapefruit juice	0.747 ± 0.098	1.00 ± 0.08

According to available literature data for samples of peel of citrus fruits, there are certain differences in the results. In a study of Ghasemi *et al*., (2009) who used percolation with methanol, the highest content of phenolics was in the orange peel (232.5 mg GAE/g), while the lowest content was found in the use of lemon peel (102.2 mg GAE/g). Moreover, the content of phenolic compound detected in juices of citruses was also lower in comparison with the literature. Petchlert *et. al.* (2013) reported the values ranging from 5.71 ± 0.01 mgGAE/mL to 10.57 ± 0.17 mg GAE/mL for mandarin and orange, respectively.

Reason for these differences might be in diverse types of extraction and solvent used, as well as the different origin of the samples.

Results of determination of total antioxidant activity using molybdenum reduction method are shown in Table 2, where the IC_{50} present the concentration of extract to reduce 50% of molybdenum cation. Values are represented as the mean taking into account the standard deviation.

Table 2: Antioxidant activity of extracts of citrus fruits.

Sample	**IC_{50} (mg/mL)**
Chlorogenic acid	3.11 ± 0.22
Orange juice	6.00 ± 0.50
Mandarin peel	9.13 ± 0.28
Orange peel	19.15 ± 0.24
Lemon peel	20.30 ± 1.98
Red grapefruit peel	24.52 ± 1.33
White grapefruit peel	30.93 ± 0.86
Red grapefruit juice	33.55 ± 0.60
Mandarin juice	36.82 ± 1.82
White grapefruit juice	39.46 ± 0.57
Lemon juice	78.11 ± 6.70

The IC_{50} values are in the range of 6.00 ± 0.50 mg/mL to 39.46 ± 0.57 mg/mL for juices, and from 9.13 ± 0.28 mg/mL to 30.93 ± 0.86 mg/mL for peels. Results are in comparable range with the IC_{50} value chlorogenic acid (3.11 ± 0.22 mg/mL) which was used as standard probe.

Since the lower IC_{50} value indicates better antioxidant activity, it might be concluded that peels of citrus fruits possess higher antioxidant activity that juices.

Presented results are in agreement with those published earlier (Ghasemi *et al*., 2009).

It is known that phenolic compounds are carriers of antioxidant activity in plant extracts (Zheng & Wang, 2001). Based on the data reported in this paper, it can be seen that there is a slight correlation between the content of total phenolics and antioxidant activity. The reason lies in complexity of isolated extracts, and as well as in the fact that antioxidant activity cannot be defined on the basis of one method used.

CONCLUSIONS

Citrus fruits contain a large variety of bioactive components and are considered as potential sources of functional components. The citrus fruits purchased in market were evaluated for the total phenolics and antioxidant activity. Presented results reveal that citrus fruits are of good quality and a valuable source of health promoting constituents.

Presented results suggest further analysis of the chemical composition of isolated extracts to identify phenolic compounds that could be responsible for the antioxidant activity, as well as more diferent asssays of antioxidant activity.

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Summary / Sažetak

Citrus je uobičajni termin za biljke iz porodice *Rutaceae.* Citrusi su važan izvor bioaktivnish spojeva koji su odgovorni za antioksidativnu aktivnost kao što su fenolski spojevi i njihovi glikozidi. U ovom radu je vršena analiza pet ekstrakata soka i kore različitih vrsta citrusa (mandarina, limun, crveni grejp, bijeli grejp i narandža) na sadržaj ukupnih fenola i antioksidativnu aktivnost. Ukupan sadržaj fenola određen je Folin-Ciocalteu metodom, dobivene vrijednosti variraju od 0.192 ± 0.015 mg EGK/mL za koru bijelog grejpa do 0.747 ± 0.098 mg EGK/mL za sok bijelog grejpa. Antioksidativna aktivnost ekstrakata ispitana je pomoću spektrofotometrijske metode bazirane na redukciji molibdena i izražena kao IC_{50}. Vrijednost IC_{50} se kreće od 6.00 ± 0.50 mg/mL za uzorak soka narandže do 78.11 ± 6.70 mg/mL za uzorak soka limuna.

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Original scientific article

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5-10

Determination of total phenolic content and antioxidant activity of ethanol extracts from *Aloe* spp.

Vidic, D. *, **Tarić, E., Alagić, J., Maksimović M.**

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Aloe spp.
phenolic;
flavonoid;
antioxidant activity;
DPPH;
ABTS

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Abstract: The ethanol extracts of leaf peel and gel of *Aloe* spp. were analyzed for their total phenolic and flavonoid profiles and screened for their antioxidant activity. The total phenolic content of the three different plant extracts and one commercial product of *Aloe vera* was determined by Folin-Ciocalteu method and flavonoid content was assessed by $AlCl_3$ method. Peel extract had the highest total phenolic content (7.99 mg gallic acid equivalents (GAE)/g extract) and flavonoid content (9.17 mg quercetin equivalents (QE)/g extract). The lowest content of phenolic and flavonoid compounds was observed in Soxhlet extract of *Aloe* gel. The *in vitro* antioxidant activity determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assays revealed that all extracts exhibited low antioxidant activity compared to quercetine and thymoquinone as standards. The best antioxidant activity had the peel extract what is in correlation with content of phenolic and flavonoid compounds.

INTRODUCTION

There are over 360 species in the genus *Aloe*. The *Aloe* genus has been used for thousands of years in the treatment of burns, wounds, skin irritations and constipation. *Aloe* has broad range of pharmacological properties, including antiinflammatory, antiviral, antioxidative actions, antibacterial, immunostimulant, antifungal, analgesic, antitumor, antidiabetic and inhibition of tumor cells activation and proliferation (Kammoun, Miladi, Ali, et.al, 2011; Nejatzadeh-Barandozi, 2013, Ray, Gupta, Ghosh, 2013).

Aloes have been used therapeutically, certainly since Roman times and perhaps long before, different properties being ascribed to the inner, colourless, leaf gel and to the exudates from the outer layers (Reynolds and Dweck, 1999). The plant is made of turgid green leaves joined at the stem in a rosette pattern. Each leaf consists of two parts: an outer green rind (skin) and an inner clear pulp (gel). *Aloe* products have long been used in health foods and for medical and cosmetic purposes. These products range from aloe drink to aloe gels, powders, capsules, creams etc. for both internal and external uses for a wide variety of indications. *Aloe vera* L. (syn.: *Aloe barbadensis* Miller) is a perennial succulent plant belonging to the Aloeaceae family. *Aloe vera* is most widely accepted and used for various medical and cosmetic purposes (Miladi and Damak, 2008).

The different species of *Aloe* have different chemical compositions. In 1851, it was discovered that potency of *Aloe* was result of aloin, a bitter juice that dried to yellow powder (Shelton, 1991). Anthraquinotes derivatives in *Aloe* vera gel play an important role in the treatment of tumors, diabetes, ulcer and cancer. Keeping this fact in view, the resent study was undertaken to isolate the phenolic anthroquinones from *Aloe* vera leaf gel (Rajendran, Narayanan, Gnanavel, 2007, Ravi, Kabilar, Velmurugan, et. al, 2011). It also has a high content of 1,8-dihydroxyanthraquinone derivatives (aloe emodin) and their glycosides (aloins), which are used as cathartic.

Phytochemical analysis revealed the presence of alkaloid, carbohydrate, tannin, steroid, triterpenoid in *Aloe vera* extracts by HPTLC method (Patel, Patel, Dhanabal, 2012). Sugar analysis of the polysaccharides after gel permeation chromatography revealed that glucose and galactose were the most abundant monosaccharide in the neutral polysaccharides from the *Aloe vera* gel juice and skin juice, respectively. The acidic polysaccharides from the two juices consisted of glucuronic acid, galactose, glucose, mannose, and xylose with variable proportions (Nejatzadeh-Barandozi and Enferadi, 2012).

The use of reversed phase high performance liquid chromatography (RP-HPLC) allowed the identification of 18 phenolic constituents. Leaf skin extracts were characterized by the abundance of catechin, sinapic acid and quercitrin. Gentisic acid, epicatechin and quercitrin were the most prominent phenolic compounds of the flowers, (López, de Tangil, Vega-Orellana, et. al, 2013).

Phenolic acids, *p*-coumaric, ferulic, homoprotocatechuic, *β*-phenyllactic, protocatechuic, caffeic, *p*-hydroxybenzoic, *p*-hydroxyphenylacetic, syringic, *o*-hydroxyphenylacetic, trans-cinnamic and vanillic acid were identified in leaves of several *Aloe* species (Nowak, Cybul, Starek, 2009).

Total phenolic content of the four different extracts of *Aloe vera* leaf skin were determined by Folin-Ciocalteu method (Miladi and Damak, 2008), and the highest content of total phenolic and best antioxidant activity were in chloroform-ethanol fraction.

There are some findings that indicate that *Aloe* gel is an effective radioprotective agent (Saini and Saini, 2011). It can be very useful in increasing the tolerance dose of radiationin cancer patients and in preventing the diarrhea which occurs during radiotherapy of pelvic and abdominal cancer as gastrointestinal tract represents one of the major dose limiting organs in radiotherapy. Many of the health benefits associated with *Aloe vera* have been attributed to the polysaccharides contained in the gel of the leaves (Hamman, 2008).

Effect of the ethanolic extract of *Aloe vera* gel on tissue antioxidants is due to reduction in blood glucose level in diabetic rats, which prevents excessive formation of free radicals through various biochemical pathways and also reduces the potential glycation of the enzymes (Rajasekaran, Sivagnanam, Subramanian, 2005).

Free radical scavenging activity of *Aloe vera* extracts get by supercritical carbon dioxide extraction (Hu, Q., Hu, Y., Xu, 2005) were compared to BHT and *α*-tocopherol as a positive probe, and the results showed that extract are stronger antioxidants.

There are a wide range of research from all over the world based upon different species of *Aloe* for antimicrobial activity (Taiwo, Olukunle, Ozor, et. al., 2005; Alemdar and Agaoglu, 2009; Lawrence, Tripathi, Jeyakumar, 2009; Khaing, 2011; Karpagam and Devaraj, 2011) and antioxidant activity (Beppu, Koike, Shimpo, et. al, 2003; Botes, Van der Westhuizen, Loots, 2008; Kumalaingsih and Wijana, 2013).

Aloe vera products have long been used in health foods for medicinal and preservative purposes. Potential use of aloe products often involves some type of processing, e.g. heating, dehydration and grinding. Processing may cause irreversible modifications to the polysaccharides, affecting their original structure which may promote important changes in the proposed physiological and pharmaceutical properties of these constituents (Ramachandra and Rao, 2008). All the investigation found that commercial soap and commercial gel samples extremely increase aloin and phenolic compounds (Ravi, et. al, 2011).

The objective of our research work was to investigate the total phenolic and flavonoid content and the antioxidant properties of the ethanol extract of peel and gel from *Aloe* spp. by radical scavenging methods including, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS).

EXPERIMENTAL

All used reagents were of the highest purity grade available and purchased from the Sigma–Aldrich Chemical Company (Germany).

Plant material

The plant *Aloe* spp. was bought at flower market. The leaves were washed with distilled water and peel was separated from the gel.

Isolation of extracts by Soxhlet extraction using ethanol as solvent was done from peel (76.0 g) S-A.p and gel (44,4 g) S-A.g, while ultrasound extraction was used for extraction of gel (28.5 g) U-A.g. Beside these samples, one commercial product of *Aloe vera* was used to compare results with crude plant extracts.

Total phenolic content

Total phenolic content of the examined extracts was determined by a slight modification of the method by Singleton and Rossi 1965. A 100 μL of sample solution, prepared in ethanol, in various concentrations was diluted with 5 mL of distilled water was mixed with 500 μL of Folin–Ciocalteu reagent, previously diluted two-fold.. After 10 minutes, 1.5 mL of 20% solution of sodium carbonate was added, and the solution obtained was diluted to 10 mL. Prepared samples were kept for 2 h at room temperature, and the absorbance was measured at 765 nm. The data were calculated according to a standard curve of gallic acid (0.5–10 μg/mL), and they were expressed as gallic acid equivalents (GAE) per gram of extracts.

Total flavonoid content

Total flavonoids in the plant extracts examined were determined by using a slight modification of the method given by Meda, Lamien, Romito, et. al, 2005. The principle of method is that aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, aluminium chloride forms acid labile complexes with the *ortho*-dihydroxyl groups in the A- or B-ring of flavonoids. A 0.5 mL of diluted extract solution was mixed with 0.5 mL of aluminium chloride (2%). After incubation at room temperature for 20 min, the absorbance of the reaction mixture was measured at 415 nm. A blank sample contained 0.5 mL of sample and 0.5 mL of distilled water. A 0.5 mL sample of aluminium chloride mixed with 0.5 mL of distilled water was used to zero the spectrophotometer. The data were calculated according to a standard curve of quercetin (3–20 μg/mL), and they were expressed as quercetin equivalents (QE) per gram of extracts.

1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity (DPPH)

The ability of the extract components to donate hydrogen atom or electron and scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined by the slightly modified method of Brand-Williams, Cuvelier, Berset, (1995). A portion of sample solution (100 μL) was mixed with 1.0 mL of 5.25×10^{-5} mol/L DPPH• in ethanol. Decreasing of absorbance of tested mixtures was monitored every 1 min for 30 min at 517 nm using Perkin-Elmer Lambda 25 UV/Vis spectrophotometer. Ethanol was used as blank, and quercetine and thymoquinone were used as a positive probe. The DPPH• solution was freshly prepared daily, stored in a flask covered with aluminum foil, and kept in the dark at 4°C before measurements. The radical-scavenging activity of the tested samples, expressed as percentage inhibition of DPPH, was calculated according to the formula

$$(\%)\mathrm{AA} = [(A_0 - A_t)/A_0] \times 100$$

where A_t is the absorbance value of the tested sample and A_o is the absorbance value of DPPH, in particular time. Percent inhibition after 30 min was plotted against concentration, and the equation for the line was used to obtain the IC_{50} value. A lower IC_{50} value indicates greater antioxidant activity.

2,2-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) radical-scavenging activity (ABTS)

The ABTS method is based on the reduction of the green ABTS radical cation (7.00 mmol/L) that was obtained by its oxidation with equal volume of potassium persulphate (2.45 mmol/L), (Katalinic, Milos, Kulisic, et. al, 2006) for 12–16 h at 4°C in the dark. On the day of analysis, the $ABTS^{\bullet+}$ solution was diluted with methanol to absorbance of 1.00 (±0.02) at 734 nm. After the addition of 100 μL of sample solution to 1.0 mL of $ABTS^{\bullet+}$ solution, decreas of absorbance was monitored every 1 min for 10 min at 734 nm using a Perkin-Elmer Lambda 25 UV/Vis spectrophotometer. Methanol was used as a blank, and quercetine and thymoquinone were used as positive probe. The radical-scavenging activity of the tested samples, expressed as percentage inhibition of $ABTS^{\bullet+}$, were calculated according to the formula

$$(\%)\mathrm{AA} = [(A_0 - A_t)/A_0] \times 100$$

where A_0 and A_t are the absorbance values of the ABTS and the test sample, at particular times, respectively. Percent inhibition after 10 min was plotted against concentration, and the equation for the line was used to obtain the IC_{50} value. A lower IC_{50} value indicates greater antioxidant activity.

RESULTS AND DISCUSSION

The yield of gel extracts were different for Soxhlet and ultrasound extraction and were 5.2% and 3.5%, respectively. Soxhlet extract of peel gave a yield of 2.4%.

Total phenolics

The total phenolic content was measured by Folin-Ciocalteu assay and expressed as mg gallic acid equivalents per gram of extract.

Table 1: Total phenolic, and total flavonoid content

Sample	**Yield (%)**	**Total phenolic mg(GAE)/g**	**Total flavonoid mg(QE)/g**
U-g	3.5	2.80±0.07	3.37±0.20
S-g	**5.2**	2.06±0.25	0.29±0.03
S-p	2.4	**7.99±0.26**	**9.17±0.19**
Av-prod	-	0.11±0.01	0.005±0.0003

U-g – Ultrasound extract of gel, S-g – Soxhlet extract of gel,
S-p – Soxhlet extract of peel

Peel extract had the highest total phenolic content (7.99±0.26 mg(GA)/g), while gel extracts had almost three times lower content for Soxhlet and four times lower for ultrasound extract (Table 1). From these results we can conclude that method of extraction had very important role, because during Soxhlet extraction degradation of thermosensitive compounds could occur. Previous results (Miladi and Damak, 2008, Kammoun, et. al, 2011) showed that content of phenolics is low in water extract (2mg(GAE)/g), while in chloroform-ethanol extract content of phenolic compounds was about 40 mg(GAE)/g.

Total flavonoids

Determination of total flavonoids is related to the formation of complex between flavonoid and $AlCl_3$ that produces a yellow colored complex. The absorbance is measured spectrophotometrically at maximum wavelength of 415 nm.

The absorbance of series of solutions with different concentration of quercetine were plotted against the yield to give a a linear calibration curve of quercetine with coefficient of correlation value of 0.9984. Flavonoid content (Table 1) was the highest in peel extract (9.17±0.19 mg(QE)/g), while the lowest was in gel extract isolated by Soxhlet extraction (0.29±0.03 mg(QE)/g).

In comparison with plant extract, commercial product of *Aloe vera* had significantly lower content of total phenolic and flavonoid compounds, 0.11±0.01, and 0.005±0.0003.

The results published by Hu, Y., Xu, Hu, Q., 2003, showed that three-year-old *Aloe vera* plant contained significantly higher levels of polysaccharides and flavonoids than two- and four-year-old *Aloe vera*, and no significant differences in flavonoid levels were found between three- and four-year-old *Aloe vera*.

Antioxidant activity

Antioxidant activity of isolated extract was determined by DPPH and ABTS methods.

The DPPH free radical scavenging activity of the *Aloe* extract was evaluated using an ethanol solution of the stable free radical, DPPH. A freshly prepared DPPH solution exhibits a deep purple color with a maximum absorption at 517 nm. This purple color disappears when an antioxidant is present in the medium. Therefore, antioxidants molecules can quench DPPH free radicals and convert them to a yellow product, resulting in a decrease in absorbance at 517 nm. Free radical scavenging activity was proportional to the concentration of the extract. Concentration of sample at which the inhibition percentage reaches 50% is its IC_{50} value. IC_{50} value is negatively related to the antioxidant activity, as it expresses the amount of antioxidant needed to decrease its radical concentration by 50%. The lower IC_{50} value, the higher is the antioxidant activity of the tested sample.

Most methods used for evaluation of radical trapping properties often utilize stable model free radicals as indicators for radical scavenging abilities, among which DPPH is recommended as easy and accurate with regard to measuring the antioxidant activity of fruit and vegetable juices or extracts (Katalinić, Milos, Modun, et. al, 2004).

Reduction power of all extracts showed that sample prepared from peel had stronger antioxidant activity than extracts of gel. Gel extracts reduced the concentration of DPPH free radical, with efficiency significantly lower than quercetin and thymoquinone, well known as good antioxidants.

The 50% inhibition of DPPH radical obtained for ethanol extracts of peel yield 45.6±5.8mg/mL. Gel extract had significantly higher concentration for 50% inhibition, and their values were 80.2±4.2 mg/mL for ultrasound extract and 558.9±55.2 mg/mL for Soxhlet extract.

The reducing ability of *Aloe* extracts on DPPH radical was determined by López *et al.,* 2013, where extract of leaf skin was more active than the flower extract. There are some data which suggest that growth stage plays a very important role ine the composition and antioxidant activity of *Aloe vera* (Hu, et. al, 2003). Etanolic and methanolic *Aloe vera* gel extracts possess maximum DPPH free radical scavenging activities (Khaing, 2011).

Among three isolated samples, the best antioxidant activity for ABTS method had extract of peel (10.4±0.5 mg/mL), while Soxhlet extract of gel (55.4±3.3 mg/mL) had the lowest antioxidant activity.

Commercial product is used in a form which one can find at the market. The best result for %AA of this sample was 20% for ABTS method and less than 10% for DPPH method.

Aloe peel is a part from aloe plant which has the best ability as antioxidant agent.

Table 2: Antioxidant activity of isolated extracts

Sample	**ABTS** **IC_{50} (mg/mL)**	**DPPH** **IC_{50} (mg/mL)**
U-g	24.1±0.7	80.2±4.2
S-g	55.4±3.3	558.9±55.2
S-p	**10.4±0.5**	**45.6±5.8**

U-g – Ultrasound extract of gel, S-g – Soxhlet extract of gel,
S-p – Soxhlet extract of peel

These results are in agreement with literature data (Ozsoy, Candoken, Akev, 2009) for antioxidant activity of aqueous extract from *Aloe vera* were IC_{50} values are significantly higher than those for ascorbic acid and α-tocopherol.

CONCLUSIONS

The antioxidant activity of isolated extracts is in correlation with the content of their phenolic compounds. The best antioxidative properties have Soxhlet extract of peel, and this sample have the highest content of phenolic and flavonoid compounds. This suggested that scavenging effect of *Aloe* extract may depend on hydrogen atom donation by the different phenolic and flavonoid compounds, and their hydrogen donor capacity, most probably accounts in large part for the antioxidant activity and may provide a basis for the pharmacological activity and therapeutic applications of this extract (Ozsoy, et. al, 2009). The activity of the extracts is not only dependent on the concentration of the phenolic compounds but also on the structure and nature of the compounds. Comparing results for antioxidant activity of samples and standards, it can be concluded that *Aloe* extracts obtained in this study could not be classified as good antioxidants.

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Summary/Sažetak

Etanolni ekstrakti kore lista i gela *Aloe* spp. su analizirani kako bi se odredio ukupan sadržaj fenola i flavonoida kao i procijenila njihova antioksidacijska aktivnost. Sadržaj ukupnih fenola u tri različita ekstrakta i jednom komercijalnom proizvodu *Aloe vera*, su određeni Folin-Ciocalteu metodom, dok je sadžaj flavonoida određen metodom sa $AlCl_3$. Ekstrakt kore je imao najveći sadržaj fenola (7.99 mg ekvivalenata galne kiseline (GAE)/g ekstrakta) kao i sadržaj flavonoida 9.17 mg ekvivalenata kvercetina (QE)/g ekstrata. Najniži sadržaj fenola i flavonoida je određen u Soxhlet ekstraktu *Aloe* gela. Antioksidacijska aktivnost je određena *in vitro* 1,1-difenil-2-pikrilhidrazil (DPPH) metodom i 2,2`-azino-bis(3-etilbenztiazolin-6-sulfonska kiselina (ABTS) metodom. Svi ekstrakti su pokazali nisku antioksidacijsku aktivnost u poređenju sa kvercetinom i timokinonom kao standardima. Najbolju antioksidacijsku aktivnost ima ekstrakt kore lista što je u korelaciji sa sadržajem fenola i flavonoida.

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Original Scientific Paper

Preconcentration of Metal Ions Using Silica Gel 60 F_{254}

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Abstract: In this study, the use of a column packed with unmodified activated silica gel as sorbent for preconcentration of Cd, Co, Cr, Cu, Fe, Mn, Ni and Pb prior to their analysis by flame atomic absorption spectrometry (FAAS) was described. Factors affecting the preconcentration of analytes such as pH, sample flow rate and volume from pure multielement aqueous solutions of known concentration, were determined. The recovery values of prepared samples of known concentration of analytes were: 87.7% (Cd), 94.3% (Co), 95.7% (Cr), 91.2% (Cu), 105.2 % (Fe), 84.5 % (Mn), 96.6% (Ni) and 101.7% (Pb), under the optimum conditions (pH; 9, sample flow rate; 6mL/min, preconcentration factor; 10). The adsorption capacity of unmodified silica gel for Cd, Co, Cr, Cu, Fe, Mn, Ni and Pb was found to be 6.05, 11.54, 13.08, 10.70, 12.18, 12.38, 11.59 and 3.28 µmol/g of adsorbent, respectively. Detection limits of the method were 4.3, 35.0, 58.2, 18.5, 37.1, 10.3, 38.8 and 55.0 µg/L for Cd, Co, Cr, Cu, Fe, Mn, Ni and Pb, respectively.

INTRODUCTION

Nowadays, the heavy metal pollution has become big problem in the world since the heavy metal in particular concentration can affect the human and living organism body (Budiman et al., 2009). Therefore, countries and communities have regulations about allowed metal ion levels in the environment. Due to these quotes, accurate determination of metal ion traces is an important field of analytical chemistry. One of the most frequently used instrumental techniques for determination of metal ion traces is flame absorption spectrometry (FAAS) due to the ease of operation and determination, low cost, high precision and selectivity (Budizak et al., 2003, Ngeontae et al., 2009, Maltez et al., 2008). Separational and preconcentrational procedures for enrichment of metal ion traces present in environmental samples are necessity before flame atomic absorption spectrometry (FAAS) determination. Reason for this pretreatment is high detection limit FAAS (or low instrumetal sensitivity) and interfering effect of sample matrix constituents on analyte (Ngeontae et al., 2007, Ngeontae et al., 2009). Main techniques for preconcentration and separation used for heavy metal traces are solid phase adsorption, solvent extraction, membrane filtration, coprecipitation, ion exchange, flotation and electrochemistry deposition (Soylak et al., 2005, Budiman et al., 2009, Takahoglu et al., 2009). For the last years, solid phase extraction (SPE) has become the most used technique for analysis of traces, mainly because it is unnescessary to use the hazardous solvents (Budiman et al., 2009). The basic principle of SPE is the transfer of analytes from the aqueous phase to the active sites of the adjacent solid phase (Dogan et al., 2007). The transfer is stimulated by the selection of optimal conditions in the system having three main components: water (liquid phase), analyte and sorbent. After sorbtion on solid phase, analyte is either desorbed by suitable solvent or the analyte with the sorbent is dissolved in a suitable solvent and then analysed. Advantages of SPE include: flexibility in selection of solid phase, low costs due to less reagent consumption, lack of emulsion, high preconcentration factor, sensitivity improval, sampling in the field, no requirements of toxic solvents, speed, simplicity and automatisation ability (Budizak et al., 2003, Tokman et al., 2003, Ngeontae et al., 2007, Budiman et al., 2009, Costa et al., 2011). Silica gel can be used as a very successful adsorbing agent, due to it′s relative inertness, good adsorption or cation exchange capability, simple modification with chemical compounds, excellent swelling resistance in different solvents, high mechanical and thermal stability (Silva et al., 2004, Budiman et al., 2009, Khosravan, 2009). Likewise, chelating agents can be bound to silica with high stability or can be chemically bound as a support which makes silica material more stable (Zougagh et al., 2005). For this reason, silica modified using organic substances which contain donor atom as S, N, P and O are of great interest (Alghami, 2012). Solid materials, like silica, are extensively used due to the presence of high number of reactive hydroxylic groups on its surface. These groups enable bonding of organic molecules which contain Lewis bases that can coordinate metal ions in solution, which increases metal ions sorbtion (Goswami et al., 2002, Tzvetkova, 2012). Among the many existing support, silica gel deserves special attention due to the fact that it is subject to chemical modification with functional compounds through of its silanol groups dispersed on the surface. The surface of silica gel is characterised by the presence of silanol groups, which are known to be weak ion-exchangers, causing low interactions, binding and extraction of ionic species (Dogan et al., 2007). Guided by this idea, aim of this work was to investigate ability of using unmodified, activated silica gel as an adsorbent (in column system) for simultaneous determination of Cr, Cu, Co, Cd, Fe, Mn, Ni i Pb from aquous samples using FAAS technique.

EXPERIMENTAL

Instrumentation

Instruments used for the experimental part of this work were: Atomic absorption spectrometer, AA240FS, Varian, pH-meter, CG 841 Schott – GERATE GmbH, analytical balance (±0.1 mg), Mettler, Toledo, Dryer, Bodalec and Havoić apparatus, type SE – 10. A glass column (150 mm height and 8 mm internal diameter) packed with unmodified activated silica gel was used for preconcentration of metals.

Chemicals and reagents

During the experimental work, the following chemicals were used: standard solutions of Cd, Co, Cr, Cu, Fe, Mn, Ni and Pb of CertiPur grade; Merck, Germany. Other chemicals were of p.a. grade: HNO_3, Carlo Erba, Milano, NaOH and $Na_3PO_4 \cdot 12H_2O$, Alkaloid, Skopje, HCl and KH_2PO_4, Semikem, Sarajevo, while $Na_2B_4O_7 \cdot 10H_2O$, KH-phthalate and silica gel 60 F_{254} were purchased from Merck, Germany.

Activation of silica gel

50 g of silica gel was added to 125 mL (1:1) of hydrochloric acid. The mixture was maintained at reflux (70-80 °C) for 6 h. After cooling, the mixture was filtered off and washed with redistilled water until no chloride appeared in the washing. The activated silica was first dried at room temperature for 48 h and then in a dryer at 110 °C for 5 h.

General procedure

The columns were sealed with small portion of glass wool, previously treated with HNO_3, to prevent material losses. Then, the columns were packed with 500 mg of activated silica gel, washed with redistilled water and conditioned with appropriate buffer solutions. Subsequently, 0.1 mg/L standard solutions of Cd(II), Cr(III), Co(II), Cu(II), Fe(III), Mn(II), Ni(II) i Pb(II), were prepared by appropriate dilution of 1000 mg/L single element atomic absorption standard solution and adjusting the pH at the optimum value. The volume of 100 mL of solutions containing previously mentioned multielement metal ions were passed through the column at a flow rate of 2 mL/min. Adsorbed analytes were eluted with 7 mL of 1 mol/L HNO_3 (Venkatesh et. al, 2004) in a volumetric flask of 10 mL. Then, the columns were washed with redistilled water (3 mL) so that the final volume of sample prior to analysis by FAAS- fast sequential technique was adjusted to 10 mL. During the process of data analysis, all values obtained were corrected by substracting the values of the procedural blank. Data are given as the mean of three replicates.

RESULTS AND DISCUSSION

Effect of pH

One of the most important factors in a liquid-solid adsorption procedure is the pH of the aqueous phase (Matoso et al., 20003). Therefore, the effect of pH on the preconcentration of trace metals on unmodified activated silica was investigated over the pH of 5 to 10 by passing 0.1 mg/L of solutions containing Cd(II), Cr(III), Co(II), Cu(II), Fe(III), Mn(II), Ni(II) and Pb(II), applying the general procedure. The experimental results shown in Figure 1 indicated that the recovery values at pH 5 were very low (<10%) for 7 of 8 investigated metal ions. The only exception is iron, with a recovery of 74.3%. According to the results shown in Figure 1 it can be concluded that the pH of 6, 7 and 8 were more satisfactory, but not quantitative for all investigated metal ions, because the best recovery of 79.9% was noted for Pb(II) ions. The exception again is recorded for iron with 101.2% at pH 6 and 94.8% at pH 8. Furthermore, it can be observed that the pH 9 was optimal for the simultaneous determination of Cd, Co, Cr, Cu, Fe, Mn, Ni and Pb ions with best recovery values recorded for Cd (87.7%), Cr (91.0%), Cu (95.9%), Mn (89.6%) and Pb (105.2%). At pH 10 a decrease in recovery values were observed for most of metal ions, probably due to hydrolysis with formation of metal hydroxides. Therefore, as a compromise between recovery values and sample pH for the adsorption of analytes on the surface of activated unmodified silica, pH 9 was chosen in subsequent work.

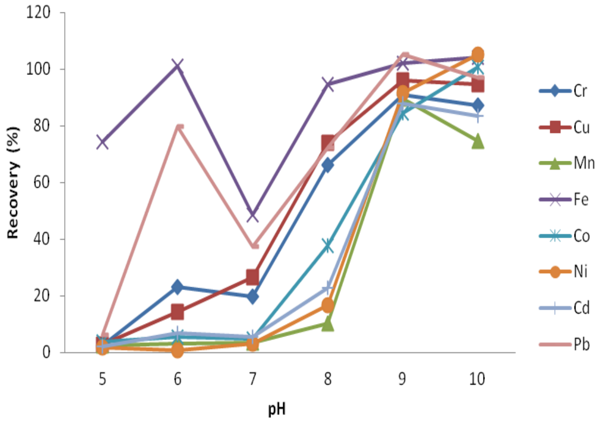

Figure 1: Effect of sample pH on the preconcentration of analytes on the unmodified silica.

Effect of sample flow rate

The sample flow rate is a very important operational parameter for the preconcentration efficiency and it determines the sample volume to be used (Maltez et al., 2008). The effect of sample flow rate on the retention of the studied metal ions on the activated silica gel was investigated by passing 100 mL of sample solution through the column and varying the flow rates between 1-6 mL/min. As it can be seen from Figure 2, the retention of Cd, Co, Cr, Cu, Fe, Mn, Ni and Pb is not significantly affected by the flow rate, indicating that the sorption kinetics is very fast. The flow rate of 6 mL/min reduces the efficiency by 10% only for copper. Therefore, the flow rate of 6 mL/min was found to be suitable and quantitative for all investigated analytes and was used for all further experiments.

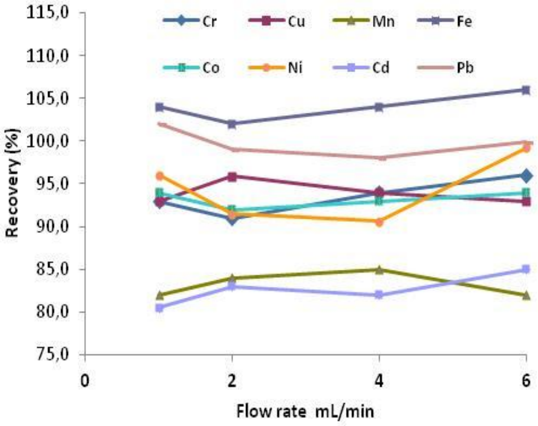

Figure 2: Effect of sample flow rate on the simultaneous retention of analytes on the unmodified silica surface. Other conditions: sample pH 9,500 mg of sorbent, analytes concentration of 0.1 µg/mL each.

Effect of sample volume

In order to obtain a high or achievable preconcentration factor of very dilute analyte solutions from large volumes, it is imperative to determine the effect of sample volume on the retention of trace metals on silica (Suleiman et al., 2007). For this purpose, 100 mL, 250 mL, 500 mL and 1000 mL of sample solutions containing 0,1 µg/mL, 0,04 µg/mL, 0,02 µg/mL, 0,01 µg/mL, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb were passed through the column at the optimum flow rate. Recovery values as a function of sample volume at the optimum flow rate and mass of sorbent (500 mg) were shown in Figure 3. It can be seen that, with the increase of the preconcentration factor from 10 to 25, 50 and to 100 a decrease of 52%, 27% and 49% in recovery value occur for most of analyzed metals, respectively. According to the results from Figure 3, quantitative recoveries were obtained for the preconcentration factor of 10. Therefore, 100 mL of sample solution was selected as the optimum volume for the preconcentration of the analytes.

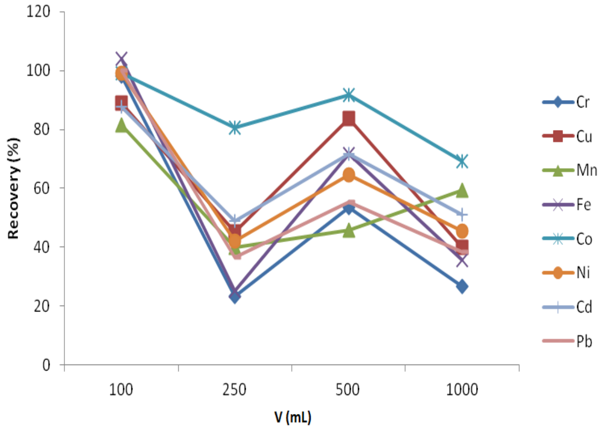

Figure 3: Effect of sample volume on the preconcentration of analytes in a column system.

Column capacity studies

The capacity of the column packed with 500 mg activated unmodified silica gel using the optimum conditions was studied by measuring the maximum quantity of the Cd, Co, Cr, Cu, Fe, Mn, Ni and Pb ions sorbed on the non-modified silica surface. For this reason, increasing quantities of Cd, Co, Cr, Cu, Fe, Mn, Ni and Pb were introduced into the column. It was possible to obtain quantitative recovery by using 0.5 mg of unmodified silica and up to 0.34 mg of Cd, Cr, Cu, Mn and Pb, Co, Fe and Ni. That means that the maximum adsorption capacity was 6.05, 11.54, 13.08, 10.70, 12.18, 12.38, 11.59 and 3.28 µmol/g of adsorbent for Cd, Co, Cr, Cu, Fe, Mn, Ni and Pb, respectively.

Analytical precision and detection limits

The precision of the method, evaluated as the relative standard deviation for solutions containing 1 mg/L Cd, Cr, Co, Cu, Fe, Mn, Ni and Pb were 3.14, 1.96, 4.60, 2.19, 4.91, 2.86, 2.64 and 1.42 %, respectively (n=12). Linear calibration graphs with correlation coefficients > 0.99 were obtained for all analyzed metals. The limit of detection (LOD) and limit of quantification (LOQ) for the eight metals were calculated as 3.3σ/S and 10σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of 12 consecutive measurements of the blank solution. Obtained results for R^2, LOD and LOQ are given in Table 1.

Table 1. Correlation coefficients (R^2), limit of detection (LOD, µg/L) and limit of quantification (LOQ, µg/L)

	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb
R^2	0.997	0.992	0.998	0.998	0.995	0.995	0.990	0.997
LOD	4.3	35.0	58.2	18.5	37.1	10.3	38.8	55.0
LOQ	13.2	106.1	176.5	56.2	112.5	31.3	117.6	166.7

Experimentally obtained recovery values for the eight metals under the optimum conditions of pH, sample flow rate and volume were shown in Figure 4. The results show that the recovery values of prepared samples of known concentration of analytes were: 87.7% (Cd), 94.3% (Co), 95.7% (Cr), 91.2% (Cu), 105.2 % (Fe), 84.5 % (Mn), 96.6% (Ni) and 101.7% (Pb).

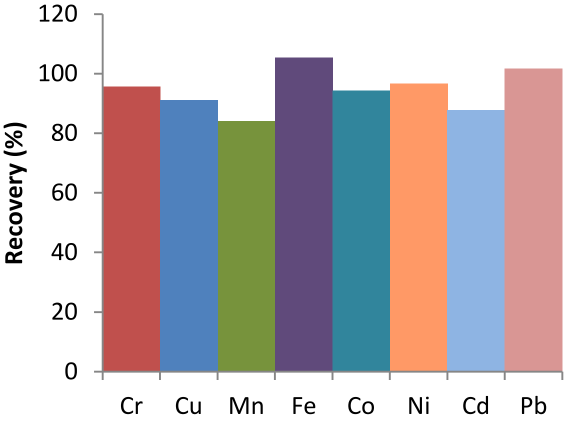

Figure 4: Results of recovery values after preconcentration on non-modified silica.

CONCLUSIONS

High values of recovery indicate that the activated silica gel 60 F_{254} can be used as: (i) an excellent sorbent for preconcentration of Fe(III), Pb(II), Ni(II) and Cr(III); (ii) a good sorbent for preconcentration of Co(II) and Cu(II); (iii) a sufficient sorbent for preconcentration of Cd(II) and Mn(II) ions from aqueous samples, under the optimum conditions obtained by this study. The use of 500 mg of the unmodified silica gel allows the preconcentration of Co, Cr, Cu, Fe, Mn, Ni and Pb by a factor of 10. Non modified silica can be reused up to 12 times. In this system, sample preparation is simple and fast, and the elution step does not involve the use of organic solvents. The method detection limits improved under the optimal conditions in the column were adequate for FAAS.

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Summary/Sažetak

U ovoj studiji opisano je korištenje kolone punjene sa nemodificiranim aktiviranim silika gelom kao sorbentom za prekoncentriranje Cd, Co, Cr, Cu, Fe, Mn, Ni i Pb, neposredno pred njihovo određivanje plamenom atomskom apsorpcionom spektrometrijom (FAAS). Određeni su faktori koji utječu na prekoncentriranje analita iz čistih multielementnih vodenih rastvora poznate koncentracije i to pH, brzina protoka i volumen korištenog uzorka. Vrijednosti *recovery*-ja pripremljenih uzoraka metala poznate koncentracije su bile: 87.7% (Cd), 94.3% (Co), 95.7% (Cr), 91.2% (Cu), 105.2 % (Fe), 84.5 % (Mn), 96.6% (Ni) and 101.7% (Pb), pod optimalnim uvjetima (pH; 9, brzina protoka uzorka; 6 mL/min, prekoncentracioni faktor; 10). Adsorpcioni kapacitet nemodificiranog silika gela za Cd, Co, Cr, Cu, Fe, Mn, Ni i Pb je iznosio 6.05, 11.54, 13.08, 10.70, 12.18, 12.38, 11.59 and 3.28 µmol/g adsorbenta, respektivno. Detekcioni limiti metode su bili 4.3, 35.0, 58.2, 18.5, 37.1, 10.3, 38.8 i 55.0 µg/L za Cd, Co, Cr, Cu, Fe, Mn, Ni i Pb, respektivno.

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Original scientific Article

Interpretation of results obtained from analyses of some raw materials for cement production, from Ribnica and Grabovica deposits

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Abstract: Basic raw materials used for portland cement clinker production are calcium, silicon, aluminium and iron oxides. In avaliable raw materials, these components are rarely found in ratio needed for clinker production. Consequently, it is important to prepare appropriate raw mixture from materials with high limestone (calcium carbonate) contents and materials with suitable contents of oxides, but with low fraction of limestone. To achieve this, it is essential to perform reliable qualitative and quantitative characterization of raw materials. In this paper, results obtained from analyses of samples used for cement production (marlstone from Ribnica and Greben quarries and marl from Greben) are presented. Chemical composition of analyzed samples is satisfactory, according to EN 197-1 standard.

INTRODUCTION

Various mineral raw materials are used in cement production, primarly limestone and marlstone followed by clay, sand, tufa, bauxite, iron ores, gypsum, etc. Some industrial wastes, such as metallurgical slag and fly ash, can also be considered as raw materials. Important factor is mineral contents needed for clinker formation or presence of some additives used in clinker production. Raw material selection depends on its availability, as well as type and desired properties of final product. Lime raw materials used for cement production are those with high content of $CaCO_3$ (40-100%) or CaO (22-56%), such as calcite, limestone and dolomite. Fraction of clay in raw material should be between 20-25 %. This component enriches basic raw material with essential quantities of acidic oxides, SiO_2, Al_2O_3 and Fe_2O_3. CaO contents in clay component is not limited, while fraction of MgO depends on its content in lime component, and should not exceed 5%. Sum of Na_2O and K_2O fractions should not be higher than 1%. Raw mixture for portland cement, which is an hydraulic binder, is composed in such manner to achieve maximum molecular saturation of acidic components (SiO_2, Al_2O_3, Fe_2O_3) with quicklime (CaO), while ensuring that there is no residual CaO in obtained clinker. Residual, chemically unbound CaO deteriorates quality of final product. Maximum content of CaO in raw material or cement is calculated from the „lime saturation factor“ equation, on the basis of percentage of acidic and basic components, obtained from chemical analysis of materials.

Best properties are obtained for cements with ratio of percentage of basic to acidic component between 1,7-2,4, i.e. in which content of basic components is two times higher than acidic components. This ratio is called hydraulic cement modulus.

Saturation factor for portland clinker varies between 90-95%. Raw materials with saturation factor between 90-98 % are considered as materials that can be calcinated without difficulties. When the saturation factor is increased, free CaO, which induce temporal instability of cement, should be considered. When the saturation factor is over 90 calcination is improved, but raw material deposits are likely to appear at the inner walls of furnace.

Raw materials with proposed hydraulic modulus, used in raw mixture for cement preparation, do not necessarily leads to quality portland cements, which are obtained if clinker contains proportions of SiO_2 to aluminum and iron oxides between 2,4 – 2,7. This value is called silica modulus and it can be used for cement quality rating.

Value of silica modulus represents the ratio of SiO_2 and sum of Al_2O_3 and Fe_2O_3 and gives ratio of calcium silicate minerals and melting minerals (calcium aluminates and calcium aluminoferites) in clinker. Cements with low values of silica modulus have higher binding rate. For more detailed quality assesment, value of alumina modulus, which represents ratio of melting minerals in clinker, is considered.

Values of alumina modulus are between 1,5 – 2,5. Alumina modulus defines composition of liquid phase in clinker. Low values are connected with low heat of hydration, slow solidification and slow binding of cement. High values of alumina modulus, together with low silicate modulus, leads to faster binding, in which case addition of gypsum is required. When the ratio of aluminium and iron oxides equals 1, alumina modulus is 0,637. In this case, only Ca_4AF (tetracalcium aluminoferrit) can be formed in clinker. This leads to formation of cement with very low heat of hydration, slow binding and negligible shrinking.

Periclase MgO

Volume staibility of cured portland cement depends, largely, on diameter of periclase (MgO) mineral phase. Clinker contains 2 % (w/w) of magnesium oxides that can be bound to clinker or can exist as free MgO. Periclase reacts with water to form $Mg(OH)_2$, in a slow reaction which continues even after all reactions that take part in solidification are finished. Volume of $Mg(OH)_2$ is higher than volume of MgO, which can lead to formation of cracks in cured cement.

When the MgO content is below 1 %, rate of cooling does not have significant impact on volume change of clinker. CaO and MgO (as periclase crystals) content should not be higher than 1 % and 5 %, respectively.

SiO_2

Nonhydraulic lime gets hydraulic properties from acidic components, and cement properties are dependent on their content. Cements with high content of SiO_2 are characterized by slow binding and hardness. High content of Al_2O_3 increases binding rate, and Fe_2O_3 makes cement resistant to corrosive chemicals.

Bases

Na_2O and K_2O react with water to form hydroxides and are a part of regular minerals used in clinker production. In contact with air, they absorb moisture and CO_2 to give carbonates. Sodium and potassium carbonates increase binding rate of cement, and they can cause adverse effects, such as fake binding. If prepared concrete is in contact with aggregate and moisture, and if alkaline content is higher than 0,6 % (given as mass of Na_2O), alkaline-silicate reaction is likely to occur.

In this work, composition of raw mixtures for portland clinker production is determined by volumetric method and X-ray fluorescence.

EXPERIMENTAL

Starting components used for preparation of raw mixtures for portland clinker were:
Marlsotne from Ribnica quarry
Marlstone from Greben quarry
Marl from Greben quarry
Pozzolan from ongoing production
Sample preparation and analysis

Marlstone and pozzolan samples are obtained automatically, in predetermined time intervals. Marlstone and marl from Ribnica and Greben quarries were sampled every twelve minutes, which gives total mass of collected sample of 5 kg per hour. Pozzolan sample is collected after grindig, but before the cyclone. Sampling is automated and performed every hour. Average sample was prepared by quartering of obtained samples, and divided on two equal parts – one for the volumetric determination of chemical composition and the other for the XRF analysis. Samples are crushed and homogenized in a laboratory mill to the specific area of 10000 cm^2/g. Tablets for XRF analysis was prepared by pressing the samples with 150 kN for 10 seconds. For XRF analysis, CubiX XRF spectrometer with Super-Q Version software was used.

Volumetric analysis of raw mixtures was performed according to EN 196-2:2005, which is current standard for this analysis.

RESULTS AND DISCUSSION

Results of chemical analysis of raw mixtures for portland cement clinker obtained with XRF and volumetric method are given in tables 1. and 2.

Table 1. *Results of chemical analysis of marlstone from Ribnica and Greben quarries*

Analyzed parameters	Marlstone, Ribnica quarry		Marlstone, Greben quarry	
	Volumetric method	XRF	Volumetric method	XRF
	Content %		Content %	
SiO_2	4,19	4,33	9,18	9,82
Al_2O_3	0,45	0,45	2,73	2,64
Fe_2O_3	0,51	0,49	2,03	1,89
CaO	52,5	52,3	46,8	46,7
MgO	0,62	0,52	0,78	0,62
K_2O	-	0,03	-	0,19
Na_2O	-	0,08	-	0,05
SO_3	0,12	0,07	0,40	0,33
Loss of ignition	41,24	-	38,00	-
Sum	99,7	-	100,0	-
Saturation factor SF	429	408,4	154,4	140,4
Hydraulic modulus HM	10,2	10,40	3,36	3,24
Silica modulus SM	4,36	6,18	1,92	2,16
Alumina modulus AM	0,88	0,43	1,34	1,39

Significant differences in composition and moduli values are obviuous for analyzed samples. Saturation factor for analyzed raw materials does not meet the requirements for portland cement. Lowest saturation factor (31,22) is obtained for marl from Greben and highest (429; 408,39) marlstone from Ribnica.

Upper limit of saturation factor, which determines maximal quantity of CaO that can be bound to acidic oxides, is 102. Thus, for raw materials with saturation factors above this limit adjustment is necessary. Presence of free CaO in cement can cause instabilities of volume, which is direct consequence of hydration of free CaO.

Another reason for preparing raw mixture with appropriate carbonate content lies in the fact that increased CaO content indicates possible release of large quantities of greenhouse gas CO_2 during the process of clinker preparation, in addition to reduced cement quality.

Marlstone samples from Ribnica quarry have highest values of silica modulus (4,36; 6,18), while samples from Greben quarry have values in the recommended limits (marlstone 1,92; 2,16; marl 2,22; 2,28). Silica modulus of pozzolan (3,02; 3,30) is slightly above the upper limit.

When silica modulus is above upper limit difficulties can occur during the calcination, which is related to the low fraction of the liquid phase and formation of higher fraction of belite. This can lead to slower binding and hardening of hydrated cement. Lowering of silica modulus leads to increased fraction of liquid phase and improves calcination of clinker.

Table 2. *Results of chemical analysis of marl from Greben quarry and pozzolan*

Analyzed parameters	Marl, Greben quarry		Pozzolan from ongoing production	
	Volumetric method	XRF	Volumetric method	XRF
	Content %		Content %	
SiO_2	27,4	27,6	13,8	13,7
Al_2O_3	8,45	8,73	3,18	2,31
Fe_2O_3	3,86	3,25	1,91	1,84
CaO	29,2	29,8	43,6	44,5
MgO	1,64	1,53	0,80	0,54
K_2O	-	0,99	-	0,29
Na_2O	-	0,04	-	0,07
SO_3	0,84	0,64	0,17	0,10
Loss of ignition	27,5	-	35,4	-
Sum	98,8	-	98,7	-
Saturation factor SF	31,2	31,7	96,8	102,5
Hydraulic modulus HM	0,73	0,75	2,32	2,49
Silica modulus SM	2,22	2,28	3,02	3,30
Alumina modulus AM	2,19	2,70	1,66	1,25

Hydraulic modulus is lowest for marl samples from Greben quarry (0,73; 0,75) and, together with the marlstone from Ribnica quarry, which have the highest values (10,2; 10,40), don't meet recommended values. These values are calculated from the results given in Table 1. and 2., because hydraulic modulus represents the rate of the lime content to the silicon, aluminium and iron oxides content.

Value of hydraulic modulus for quality cements is 2. Cements with HM < 1,7 usually don't have satisfactory strenght, while those with HM > 2,4 don't have constant volume. Raw materials with high hydraulic modulus need more heat for calcination, have higher strenght and heat of hydration, while chemical inertness is decreased.

Alumina modulus of analyzed samples in general does not meet the recommended values for portland cement, except for marl from Greben quarry. Lowest value (0,88; 0,43) was determined for marlstone sample from Ribnica quarry. This value is closest to the value at which tetracalcium aluminoferrites form (0,637). Such low value of alumina modulus is consequence of low content of aluminium and iron oxides in analyzed sample. Alumina modulus has crucial role in liquid phase formation in clinker. AM value of 0,637 means that molecular proportion of aluminium and iron oxides is 1 which, during the calcination, exclusively leads to formation of $4CaO{\cdot}Al_2O_3{\cdot}Fe_2O_3$ (tetracalcium aluminoferrite). Clinker obtained from this raw material can not contain $3CaO{\cdot}Al_2O_3$ (tricalcium aluminate). These cements are charaterized by very low heat of hydration, slow binding and negligible shrinking. Cements with high binding rate have high values of alumina modulus and low values of silica modulus. Thus, rate of binding can be regulated by addition of gypsum.

MgO content

Presented results indicate that analyzed raw materials have low content of MgO, and his presence will not affect cement quality.

Sulfur S-SO_3

Sulfur is usually found in raw materials for cement clinker production, in the form of pyrite and marcasite. Also, it can enter in raw material from the fuel, during the calcination.

During the process of clinker calcination, sulfur contained in raw material and fuel converts to sulfur dioxide, which can react with fly ash and oxygen to form volatile sulfates that condense on raw material in cooler parts of furnace.

Evolution of SO_2 begins in preheating zone, where it reacts with $CaCO_3$ to form $CaSO_4$, which partially decomposes to SO_2 and CaO in calcination zone and causes increase of SO_2 in circulating gases.

Sulfates of alkaline metals in clinker improve starting hardenss of cement. However, increased sulfur content in the raw material as well as in the fuel can cause increased content of SO_2 in waste gases. For regulation of binding rate it is necessary that cement contain defined quantity of calcium sulfate, which is added as the correcting agent in portland cements. Maximum allowed content of SO_3 which will not cause sulfate swelling of cement is between 2,5 and 4 %.

Results of the analysis show that this component is far below recommended values, which implies that fuel with higher content of sulfur can be used, and that addition of gypsum can be considered.

In most cases, good agreement between results obtained by volumetric and XRF methods are obtained.

CONCLUSIONS

Analyzed samples used in cement production (marlstone from Ribnica and Greben quarries and marl from Greben) meet the recommended values according to EN 197-1, but can not be used as individual components for cement production. Preparation of appropriate raw mixtures is possible from these components.

Results also show that chemical composition of analyzed samples is well defined and that there is low content of impurities.

Raw mixtures for cement production can be prepared by mixing of appropriate quantities of particular material, with addition of corrective agents, such as iron ore (Fe_2O_3), up to the 1 % and industrial fly ash from thermoelectric plants, up to the 5 %.

Because of different composition and mass fraction of components required for portland cement production, constant analytical monitoring with calculations of required quantities of particular components for raw mixture is needed.

In general, raw mixture for cement clinker must contain 75 % of $CaCO_3$ and 25 % of other components, mainly SiO_2, Al_2O_3, Fe_2O_3. Detrimental components are MgO, K_2O, Na_2O and SO_3.

There is no significant difference between results obtained by volumetric and XPS method. Choice of method does not have impact on process monitoring and calculations of raw material quantities. However, it is crucial for time saving, as XRF method is considerably faster.

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Summary / Sažetak

Osnovne sirovine koje se koriste za proizvodnju portland cementa su kalcij, silicij, aluminij i željezo oksid. Važno je pripremiti odgovarajuću smjesu sirovina od materijala sa visokim sadržajem krečnjaka (kalcijum karbonata) i materijala sa odgovarajućim sadržajem oksida. Da bi se to postiglo, neophodno je da se izvrši pouzdana kvalitativna i kvantitativna karakterizacija sirovina. U ovom radu, dobiveni rezultati iz analize uzoraka se koriste za proizvodnju cementa (laporac iz Ribnice i kamenolom i lapora iz Grebena). Hemijski sastav analiziranih uzoraka je zadovoljavajući, u skladu sa EN 197-1 standardom.

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Fundamental thermal concepts understanding: the first-year chemistry student questionnaire results

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Abstract: The main aim of this study is to show the research results of learning outcomes on thermal physics that have gained a group of first-year chemistry students in the fall semester of the academic 2013-2014 year. In this paper is described the use of a questionnaire to explore chemistry freshmen capacity to which they achieve their understanding of thermal physics concepts they learn within the chemistry and physics courses during the first study year. A used questionnaire contains 20 questions including a question on which the 94 study participants had the task to give their explanations related to a selected question. Research results show that knowledge about thermal equilibrium, the first law of thermodynamics and other relations among physical quantities students did not acquire in a way to present their scientific literacy developed enough at university learning outcomes level. Students need some additional instructions to find their pathways from pre-instructional alternative concepts to learn the scientific concepts through new working sessions enriched with experiments and demonstrations by instructors, teaching assistants as well as a group of students actively involved in learning on thermal physics subject matter.

INTRODUCTION

One of main concepts within the chemistry study curriculum is thermal physics concept as fundamental one that students study within the first-year science courses (General Chemistry and General Physics). David Meltzer (2005) highlighted the importance of fundamental thermal physics concepts to avoid confusion associated with students' difficulties to understanding dependent or independent quantities of state and process (heat, internal energy, entropy, and work). Meltzer has conveyed several reasons why students could express some difficulties caused by a confusion gained in mechanics to understand energy and work concepts, or in using models or approximations in physics. Talking about known Sommerfeld's story and a view of thermodynamics as a difficult subject matter Rodrigo de Abreu and Vasco Guerra (2012) have given the framework of a need to define and to introduce all basic concepts on thermodynamics to be understandable for learners. They presented „a simple and clear model" to introduce thermodynamics, and its scientific concepts of heat, internal energy and work formulated in the frame of laws of thermodynamics. Investigation of students' understanding of thermal physics concepts has been conducted by a group of authors who found evidences of student difficulties „in applying scientific concepts in everyday contexts", according to received the conceptual test answers (Chu et al., 2012).

In this paper, we describe the use of a questionnaire to explore chemistry freshmen capacity to which they achieve their conceptual understanding in thermal physics they learn within the chemistry and physics courses during the first study year semester of the academic 2013-2014 year.

The study objective is concerned with misconceptions in thermal physics and low students' success in a partial *Physics I* examination. In this sense, the specific objective of this study was to discover whether students have integrated previously acquired knowledge with knowledge gained in general chemistry and general physics courses at the university level in the field of thermal physics trying to find a way for changing their misconceptions. An insight in students' thermal physic learning outcomes is important for their further education in chemistry as their field of study at university.

The significance of research findings could initiate findings of better teaching-learning methodology to enable students to take part in an active learning environment focused to change their passive role in the traditional *ex cathedra* learning environment. Among other things, this means that it could launch initiatives to better organize the curriculum in the first year of chemistry study with at least two smaller student groups (less then 60 students in each group) that could attend classes aimed toward achieving better understanding and the necessary scientific literacy.

Literature background

Several widely used questionnaires that cover the scientific concepts, developed by science researchers have been used in different disciplines such as mechanics, astronomy, electromagnetism, optics, thermal physics (Hestenes, Wells, & Swackhamer, 1992; Zeilik et al., 1998; Zeilik, 2002; Sadler et al., 2010; Hieggelke et al., 2001; Prince, Vigeant & Nottis, 2012; Yeo & Zadnik, 2001). A questionnaire developed in Australia by Shelly Yeo and Marjan Zadnik (2001) was evaluated and their research results were presented after its application. Their final research instrument (The Thermal Concept Evaluation/TCE) consists of the 26 questions for reaching students' knowledge related to the "real-life" understanding of phenomena and environmental issues according to the basic learning thermal concepts. Yeo and Zadnik discussed the response choices by students' alternative conceptions and beliefs. The authors evaluated their research instrument by comparison of student scores reached by upper and lower group of students. Yeo and Zadnik presented the average normalized gain (g factor) between the pretest and posttest scores in amount of 0.30. The TCE can be used for testing the thermal conceptual understandings at high school and university first-year study level.

Prince, Vigeant, and Nottis (2012) conducted a research to explore undergraduate engineering student misconceptions related to: temperature vs. energy, temperature vs. perceptions of hot and cold, factors that affect the rate vs. amount of heat transfer, and thermal radiation using the Heat and Energy Concept Inventory (HECI). They found from the HECI research data that student misconceptions were resistant to change if standard instructions were implemented. For students' conceptual changes they highlighted a need to introduce an ontology training and implementation of the inquiry-based activities.

Katharyn Nottis with two colleagues (2010) explored student misconceptions about heat transfer that were found „even after students successfully completed relevant coursework". They studied possibilities in students' conceptual changes after inquiry-base activities and application of a concept inventory with ten questions conducted for a group of chemical engineering students. The research results showed the increasing scores after new learning activities that were reached by inquiry-based learning (the simulations).

Heat and temperature were the research subjects conducted by Paul Jasien and Graham Oberem (2002) in the undergraduate and post-baccalaureate student groups. Jasien and Oberem created and implemented a survey related to the heat and temperature applying a multiple choice question form. Their sample was the 30 students at two universities in California. Jasien – Oberem's research results showed that the research participants had „confusion about a number of concepts" and knowledge less then it was expected. Lack of understanding was a main characteristic of the findings among students who took part in the Jasien-Oberem survey, especially for topics such as the thermal equilibrium problems, specific heat, heat capacity, heat transfer and temperature change.

Several resistant misconceptions in thermal physics Pizzolato and collaborators were investigated within a research conducted among engineering students who worked in groups and performing scientific investigations using materials to practice the „collecting, processing and analyzing data" that enabled them to enhance their practical and reasoning skills (Pizzolato et al., 2014). This study is an affirmation of an inquiry-based teaching approach. The research findings showed that students improved their conceptual knowledge, practical and reasoning skills in amount of 55% of reduction of unanswered questions compared with questionnaire results in pre and post answering sequences related to the conceptual questions in thermal physics.

What students need to know?

Students enrolled to study chemistry and aiming to become scientists, engineers or chemistry teachers cross into new intellectual domain after their secondary schooling. Their prior knowledge is considered as a basic one that enables them during the first-year of study to develop deeper and wider scientific concepts in three main fields: general chemistry, general physics, and mathematics. For chemistry freshmen, the most important idea is to be familiar with units of measurements as main concepts defined in the Standard International System (SI). Other essential ideas to develop are concepts of force, energy, work, pressure, temperature, heat, and entropy. Chemistry students should understand the laws of thermodynamics and the nature of interactions between atoms, molecules, and other ensembles of particles.

Chemistry freshmen study several topics on thermal physics within the *General Chemistry* and *General Physics* syllabi that are considered as very important ones for their study field. For chemistry students, the thermodynamics knowledge is fundamental to „understand the behavior of materials under various environmental conditions and to develop process for novel materials" (Balducci et al., 2009, p.305).

Chemistry freshmen need to understand thermal physics concepts in a such a way so eventually they possess knowledge to solve a thermal physics questionnaire, applied under this study, which contains the questions about:

temperature,
internal energy,
heat and specific heat,
phase changes,
system work,
the first low of thermodynamics,
isothermal process,
constant volume (pressure) process, and
adiabatic process.

It is also important that students have to show their factual knowledge about relationships among thermal physics quantities. The temperature and thermal equilibrium concepts are important knowledge content and their understanding of the substance phase changes, and understanding of energy in the form of heat released or absorbed during a change of substance phase is important. It should be noted that chemistry freshmen need to know the meanings of the laws of thermodynamics applied in everyday context. According to the essential thermal physics concepts students need to make a distinction between thermal physics quantities by their nature.

METHODS

Participants

Study participants were the 94 first-year chemistry students at Sarajevo University in Bosnia and Herzegovina. A dominant number of students were females (82% of students under research). The study was carried out in the fall of 2013-14academic year at the end of semester. They were enrolled to study chemistry without a university-entrance examination.

Table 1: Distribution of students by residence.

Residence	Frequency	Percent
Sarajevo Canton	36	38.3
The Federation of BH	53	56.4
The Republic of Srpska	4	4.2
International student	1	1.1

The students were from various regions of Bosnia and Herzegovina attending different secondary schools before their university study. Their distributions by residence and secondary school are presented in Table 1 and Table 2.

Table 2: Distribution of students by secondary school.

Secondary school	Frequency	Percent
High school	67	71.3
Medical secondary school	24	25.5
Technical secondary school	3	3.2

The students under this study had just started their first semester at university, taking six courses, all as required ones: Mathematics I, Physics I, General Chemistry I, Stehiometry, Mineralogy with Crystallography, and Introduction to Chemistry Laboratory. The students completed different secondary school curricula Because attending different secondary schools, these students had studied physics differently, according to the number of study years in physics class (from one to four school years). They completed their secondary education in three types of secondary school: a high school, medical secondary school, and technical secondary school (mechanical and agricultural).

Research instruments

To measure the level of student understanding of subject of thermal physics we used the Thermal Concept Questionnaire (TCQ) as a research instrument. It is a set of 20 questions (see Appendix) aimed to explore student understanding of the basic concepts on thermal physics. The first 12 questions were adapted ones from a research instrument created in Australia known as the Thermal Concept Evaluation (Yeo & Zadnik, 2001). Next two questions are adapted from a survey found in a paper by Jasien and Oberem (2002), and the rest are six questions according to the Murray's quiz (2012) that is an *online* material available for thermal physics knowledge testing.. The TCQ composition consists of five subscales to cover t subjects of (1) heat transfer and temperature changes; (2) boiling; (3) heat conductivity and equilibrium; (4) freezing and melting; (5) the internal energy-heat-work relationships. TCQ was applied once during this diagnostic study of students' conceptual knowledge on thermal physics. As was mentioned, a main TCQ usage is related to evidences relevant for a physics course development, assessment and instructor awareness of student learning difficulties in the introductory physics course (*Physics I*). The *Physics I* syllabus realization is seen by the course instructors as a main difficulty in the teaching-learning process according to thermal physics topics that take only two weeks with only six teaching-learning hours, each 45 minutes long.

Evaluation of the student TCQ achievements, were based on a conducted scoring by authors. Each correct student's TCQ answer was graded with one point (20 points in total) plus six points as maximum of the bonus points for each correct explanation and calculation related to the Q6. The scoring rubric elements for achieving these six points are:

A (6 points): a notion of the thermal equilibrium given textually, the thermal equilibrium equation expressed correctly, correct calculation of required temperature, and correct answer chosen;
B (4 points): the thermal equilibrium equation expressed and chosen answer correctly;
C (1 point): a notion of the thermal equilibrium, and correct answer chosen.
D (1 point): a description of the thermal equilibrium without the equation, and answer chosen correctly.
E (0 points): without or no logical explanation.

RESULTS AND DISCUSSION

A group of the 94 chemistry freshmen among 110 of students taking the *Physic I* class were tested about understanding of basic thermal physics concepts using the TCQ. This questionnaire was realized during the *Physic I* class (45 minutes long) by students who took part in examination voluntarily. All of them gave their names to receive some extra bonus; 0-6 points within the *Physics I* grading scheme. Students' ranking statistics is presented in Figure 1 according to their TCQ correct answers. The TCQ questions and percents of each Q1-Q20 item frequencies are presented in Appendix Table 4.

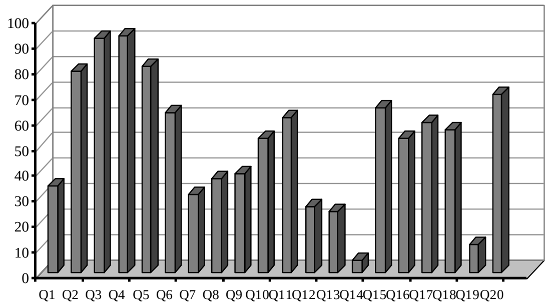

Figure 1: Percents of correct answer frequencies for each TCQ question.

The Table 4 in Appendix and Figure 1 revealed that around 60-90% of students perceived scientific knowledge of a body/system temperature (see the data of Q2-Q6 and Q20 answers). The majority of students (Q14; 95% and Q19; 89%) have no developed concepts and understanding of causal relations for both thermal energy (internal energy) change and heat flow. The data show that a number of students selected the following answers Q1: b; Q14: b, c, d and Q19: c (Table 4 in Appendix). It indicates that chemistry freshmen have no factual knowledge about temperature, heat and internal energy.

Students' results were tested for normality of distribution with Kolmogorov-Smirnov and Shapiro-Wilk tests related to the three variables: (1) TCQ student score; (2) studentgender, and (3) usage of textbooks as learning sources according to the *Physics I* syllabus. The points' distribution by students was not normal; the scores were expressed as median and compared with Mann-Whitney test for different groups. Chemistry freshmen reached the median value of 10 points for both females and males. A significant difference was not found in scores between male and female students (Mann-Whitney U: 621.0, p = 0.74) or between students according to the textbooks used or not (Mann-Whitney U: 761.5, p = 0.65). The minimum score was four points (2 % of students) achieved by the male students, whereas the female students have got a minimum score of three points (only one student). Two female students achieved a maximum score of 17 points (2 % of students), and 15 points was the maximum score accomplished by one male student.

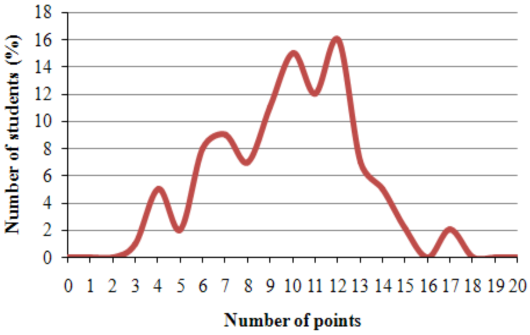

Figure 2: Students' distribution by points achieved.

Students' distribution by number of achieved points according to the Q1-Q20 answers without bonus points is presented in Figure 2. Majority of students (15% and 16% of them) achieved 10 and 12 points, respectively, showing a bimodal statistics. The distribution of the students among the above mentioned groups is shown in Figure 3. The students showed undeveloped scientific concepts but hold some alternative concepts.

Students were classified into three groups according to the number of achieved points as follows:

Group 1: 3 – 10 points
Group 2: 11 – 15 points
Group 3: 16 – 20 points

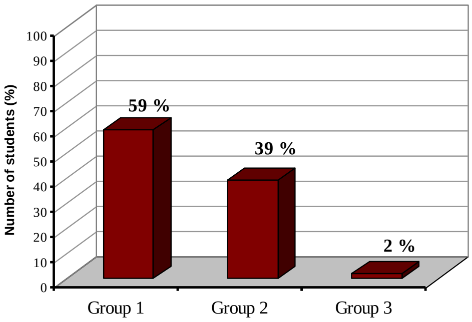

Figure 3: Students' distribution by group category.

There are several examples to show students' alternative conceptions within their incorrect answers.

1. According to the Q1-d 3% of students considered that „*the temperature of an object depends on its size*".
2. The answer Q9-c was chosen by 13% of students who considered that „*different materials hold the same amount of heat*".
3. The Q10-a answer was chosen by 22% of students who had thought that „*temperature can be transferred*".

According to the data seen in Table 4 one can notice that Q14 was the most difficult question (5% of students with correct answers). The same situation is found with the Q19 (only 10% of students gave their correct answers). The highest number of students with correct answers was achieved according to the Q4 and Q5 (more then 80% of students).

Distribution of points by each student (marked by their code number) is presented in Figure 4.

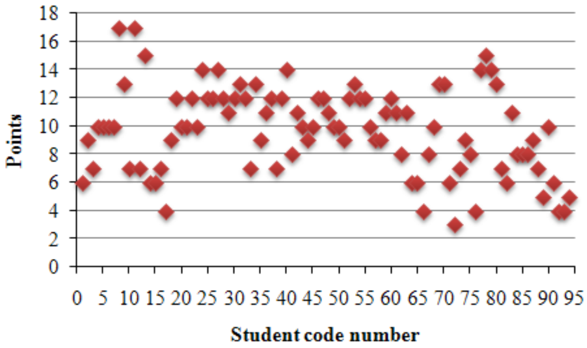

Figure 4: Points' distribution by each student (N = 94).

The passing points in accordance to the university threshold was 11. The TCQ passing score (count > 11 points) have achieved 40% of students. It is important to note that 60% of students showed a low examination score on thermal physics concept understandings and knowledge applications in everyday situations. If one counts the overall results it can be found that study participants have won only 49% of the potential 1,880 points.

Bonus point results

Students could earn from one to the six bonus points giving their explanations to one of the Q6 answer choices. Total number of students who presented their explanations was 47 (50%), but accounted points were found for 33 (36%) of students with bonus scores.

Table 3: Bonus points results.

Category	Bonus points	Number of students (%)
A	6	0
B	5	0
C	1	26 (28%)
D	1	7 (8%)
E	0	14 (15%)

Therefore, according to the aforementioned coding scheme students' bonus point results are presented in Table 3. As can be seen from these data, none of the students gave an answer in the A or B bonus point category. The other half of students had presented their explanations, but other half did not try to give any word or equation to explain their reasons for a chosen answer.

Students' explanations according to the Q6 answer

Students were asked to give their explanations for the chosen the Q6 answer. Mostly student explanations were not precise. Students typically did not express the temperature unit (degree Celsius). They only used digits (40, 30, 10 or 25). The calculations were presented without proper equation or notion about temperature equilibrium. Several examples of students' explanations of the chosen Q6 answers are presented:

Example 1: Q6 *Answer c) and explanation as*

$$Q_1 = Q_2$$

Example 2:

Q6 *Answer c) and explanation by a calculation as*

$$t = \frac{40+40+10}{3} = 30$$

Example 3:

Q6 *Answer b) and explanation by a calculation as*

$$t = \frac{t_1 + t_2}{2} = 25$$

Example 4:

Q6 *Answer c) and explanation by a calculation as*

40 – 10 = 30

Example 5:

Q6 *Answer c) with following explanation:*

Mixing water at 40 heat transfer starts from warmer to colder water at 10.

Example 6:

Q6 *Answer c) with following explanation:*

Mixing water at 40°C with water at 10°C its internal energy (of hot water) will decrease and of cold water will increase to reach water mixed at 30°C.

From these examples, one can see that the student explanations were not scientifically based or not given by arguments as was expected by university students. In the other hand, if they have used the thermal equilibrium equation in general form, they did not know to articulate meaning of each Q in such equation (see Example 1).

One may ask why 50% of students did not give any argument or a try to provide an explanation. One can find an answer in the teaching/learning approach. Firstly, the study participants attended their classes in a large group of students (approximately 100 students at every class meeting) where they could not participate as active learners in *Physics I* and *General Chemistry I* course because of an inadequate working space (a classroom in the form of an amphitheater). Secondly, in such learning environment they attended only two meetings each 135 minutes long with the *Physics I* instructor to learn about thermal physics concepts mostly instructor-centered. Thirdly, as the most important reason can be found in a fact that about 50% of students who learned the same concepts in secondary school two or three years ago, have still retained many misconceptions.

It is significant to point out that, despite requests by researchers that students should give their answers; they found a total of 3.8% of the unanswered questions in the questionnaire. For these students, it was not possible to determine the existence of misconception and then there is no strategy for conceptual change.

CONCLUSION

The prior and actual thermal physics learning outcomes of chemistry freshmen examined in this study showed the gap in conceptual understanding of thermal physics. The findings show that around 60% of chemistry freshmen have not presented even their factual knowledge. The research participants did not demonstrate the required knowledge outcomes on thermal physics. Students' learning outcomes on thermal physics need to be appropriate for individuals who need to learn more complex subject matter in Physical Chemistry course and other chemistry courses at higher study years.

These study findings show that students under research had difficulties in understanding heat, temperature and phase changes similar to students under mentioned study results. The same confusion as Jasien and Oberem (2002) highlighted were found among these study results about:

(1) the meaning of thermal equilibrium;

(2) the physical basis of heat transfer and temperature change;

(3) the relationship between specific heat, heat capacity, temperature change, work-heat-internal energy relationships, and phase changes.

Students who were choosing the incorrect TCQ answers have showed “the beliefs of naive thinkers“, as Yeo and Zadnik commented in their paper (2001). Following the Yeo and Zadnik instructions, we think that a detailed examination of student alternative concepts can be carried within an extended research. It should be a basic strategy of the *Physics I* syllabus realization using research results related to the thermal physics subject matter, but associated with a need for additional class hours

Hiebert and Lefevre (1986) thought that “knowledge may be a collection of unrelated facts, whereas conceptual knowledge puts the focus on relationships“can be confirmed by data presented in Table 4. One can see there are students’ misconceptions and lack of factual knowledge according to aforementioned learning thermal physics topics. Following ideas proposed by Reinders Duit (1999) a physics instructor need to help students to find their pathways toward the conceptual changes from „pre-instructional conceptions“ to the science concepts that need to be learned. It is important to note that science instructors in the same time need to learn how to develop conceptual change strategies for their students within a new teaching practice different then traditional one (Duit, 1999; Duit & Treagust, 2003; Kalman, Rohar & Wells, 2004).

In conclusion, it is evidently that chemistry freshmen need tto gain better factual knowledge and to overtake a very complex and difficult process to accomplish their conceptual changes of thermal physics understanding. Students need some additional instructions to find their pathways from pre-instructional alternative concepts to learn the scientific concepts through new working sessions enriched with experiments and demonstrations by instructors, teaching assistants as well as students as active learners.

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APPENDIX

THERMAL CONCEPT QUESTIONNAIRE (TCQ)

Table 4: Questions and answer statistics (N =94)

Q1. What is the most likely temperature of ice cubes stored in a refrigerator's freezer compartment?	Percents of frequency
a) –10°C	34
b) 0°C	61
c) 6°C	0
d) It depends on the size of the ice cubes.	3
No answer	2
Q2. The ice cubes Edin left on the counter have almost melted and are lying in a puddle of water. What is the most likely temperature of these smaller ice cubes?	Percents of frequency
a) –10°C	0
b) 0°C	79
c) 6°C	13
d) 10°C	6
No answer	2
Q3. On the stove is a kettle full of water. The water has started to boil rapidly. The most likely, temperature of the water is about:	Percents of frequency
a) 86°C	0
b) 98°C	92
c) 112°C	2
d) none of the above answers could be right.	5
No answer	1
Q4. Five minutes later, the water in the kettle is still boiling. The most likely temperature of the water now is about:	Percents of frequency
a) 86°C	0
b) 98°C	93
c) 112°C	6
d) 120°C	0
No answer	1
Q5. What do you think is the temperature of the steam above the boiling water in the kettle?	Percents of frequency
a) 86°C	11
b) 98 °C	81
c) 112 °C	4
d) 120 °C	1
No answer	3
Q6. Maja takes two cups of water at 40°C and mixes them with one cup of water at 10°C. What is the most likely temperature of the mixture?	Percents of frequency
a) 20°C	1
b) 25°C	28
c) 30°C	63
d) 50°C	2
No answer	6

Give here your explanation for the chosen answer.

Q7. Selim believes he must use boiling water to make a cup of tea. He tells his friends: “I couldn’t make tea if I was camping on a high mountain because water doesn’t boil at high altitudes.” *Who do you agree with?*	Percents of frequency
a) Adi says: “Yes it does, but the boiling water is just not as hot as it is here.”	31
b) Amra says: “That’s not true. Water always boils at the same temperature.”	33
c) Ema says: “The boiling point of the water decreases, but the water itself is still at 100^0C.”	16
d) Tin says: “I agree with Selim. The water never gets to its boiling point.”	17
No answer	3

Q8. Samir takes a can of cola and a plastic bottle of cola from the refrigerator, where they have been overnight. He quickly puts a thermometer in the cola in the can. The temperature is 7^0C. What are the most likely temperatures of the plastic bottle and cola it holds?	Percents of frequency
a) They are both less than 7^0C.	11
b) They are both equal to 7^0C.	37
c) They are both greater than 7^0C.	13
d) The cola is at 7^0C but the bottle is greater than 7^0C.	35
e) It depends on the amount of cola and/or the size of the bottle.	3
No answer	1

Q9. Aida asks one group of friends: “If I put 100 g of ice at 0^0C and 100 g of water at 0^0C into a freezer, which one will eventually lose the greatest amount of heat? *Which of her friends do you most agree with?*	Percents of frequency
a) Emina says: “The 100 g of ice.”	4
b) Lada says: “The 100 g of water.”	39
c) Luka says: “Neither because they both contain the same amount of heat.”	13
d) Nermin says: “There’s no answer, because ice doesn’t contain any heat.”	7
e) Emil says: “There’s no answer, because you can’t get water at 0^0C.	24
No answer	13

Q10. After cooking some eggs in the boiling water, Meliha cools the eggs by putting them into a bowl of cold water. Which of the following explains the cooling process?	Percents of frequency
a) Temperature is transferred from the eggs to the water.	22
b) Cold moves from the water into the eggs.	18
c) Hot objects naturally cool down.	5
d) Energy is transferred from the eggs to the water.	53
No answer	2

Q11. Why do we wear sweaters in cold weather?	Percents of frequency
a) To keep cold out.	1
b) To generate heat.	5
c) To reduce heat loss.	61
d) All three of the above reasons are correct.	21
No answer	12

Q12. Damir is reading a multiple-choice question from a textbook: “Sweating cools you down because the sweat covering your skin:	Percents of frequency
a) wets the surface, and wet surfaces draw more heat out than dry surfaces.”	4
b) drains heat from the pores and spreads it out over the surface of the skin.”	28
c) is the same temperature as your skin but is evaporating and so is carrying heat away.”	41
d) is slightly cooler than your skin because of evaporation and so heat is transferred from your skin to the sweat.”	26
No answer	1

Q13. As a material freezes, it	Percents of frequency
a) decreases in temperature and transfers heat to the environment	52
b) increases in temperature and absorbs heat from the environment	0
c) remains at the same temperature and transfers heat to the environment	24
d) remains at the same temperature and absorbs heat from the environment	6
e) none of the above	16

No answer	1
Q14. If body A (temperature 30^0C, mass 2g, specific heat 4 J/g^{0}C) and body B (temperature 30^0C, mass 25 g, specific heat 2 J/g^{0}C) are placed in contact	Percents of frequency
a) heat energy will naturally flow from B to A since B has the larger mass.	6
b) heat energy will naturally flow from A to B since A has the larger specific heat.	24
c) heat energy will naturally flow from B to A since the product of the mass and specific heat is larger for B.	22
d) heat will naturally flow from A to B since A has more heat per gram than B;	34
e) no heat energy will flow.	5
No answer	7
Q15. During an isothermal process then a gas internal energy change is:	Percents of frequency
a) positive	10
b) negative	12
c) zero	65
d) undecided	11
No answer	2
Q16. If the gas expands in an isothermal process, then heat is	Percents of frequency
a) positive	53
b) negative	22
c) zero	7
d) undecided	12
No answer	6
Q17. If the gas is compressed in, then the work done by the gas is	Percents of frequency
a) positive	21
b) negative	59
c) zero	7
d) undecided	10
No answer	3
Q18. If the volume of the gas decreases (P is constant), then the work done by the gas is	Percents of frequency
a) positive	19
b) negative	56
c) zero	11
d) undecided	11
No answer	3
Q19. During an adiabatic expansion of a gas its change in internal energy is	Percents of frequency
a) positive	14
b) negative	11
c) zero	59
d) undecided	15
No answer	1
Q20. If the temperature of the gas increases then its change in internal energy is	Percents of frequency
a) positive	70
b) negative	19
c) zero	4
d) undecided	5
No answer	2

Summary/Sažetak

Glavni cilj u ovom radu je da se pokažu rezultati istraživanja ishoda učenja o kalorici i termodinamici koje su stekli studenti prve godine studija hemije u zimskom semestru akademske 2013./2014. godine. U ovom radu je opisano korištenje upitnika za istraživanje kapaciteta studenata prve godine studija hemije o njihovom razumijevanju osnovnih koncepata iz kalorike i termodinamike, koje su stekli u okviru predmeta opće hemije i opće fizike. Korišten je istraživački instrument koji sadrži 20 pitanja na koja su 94 studenta davali odgovore, uključujući i jedno pitanje na koje su imali zadatak da daju objašnjenje za odabrani odgovor. Rezultati istraživanja pokazuju da studenti nisu stekli odgovarajuće znanje o toplinskoj ravnoteži, prvom principu termodinamike i drugim odnosima među fizičkim veličinama iz kalorike i termodinamike, pokazujući nedovoljno razvijenu naučnu pismenost za univerzitetski nivo ishoda učenja. Studentima su potrebne dodatne instrukcije kako bi našli načine da prevladaju prethodne alternativne koncepte ka usvajanju naučnih koncepata, kroz aktivnosti obogaćene eksperimentima i demonstracijama od strane nastavnika, asistenata kao i grupe studenata aktivno involviranih u savladavanje gradiva iz kalorike i termodinamike.

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DFT Study and Biological Activity of Some Methylxanthines

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Abstract: Using quantum chemical methods (density functional theory level), biologically active methylxanthines were investigated. All calculations were performed at B3LYP/6-31G* level of theory. The electronic chemical potential, highest occupied molecular orbital (HOMO)/lowest unoccupied molecular orbital (LUMO) gap, dipole moment, vibration frequencies, electrophilicity, chemical hardness and logP were calculated. Obtained results correspond well with some of the pharmacological properties.

Calculated properties could be useful for quantitative structure-activity relationship (QSAR) analysis which will be performed in the future.

INTRODUCTION

Methyl derivatives of xanthine are a group of alkaloids commonly used for their effects as mild stimulants on various organ systems such as cardiovascular and CNS (Central Nervous System), and skeletal muscles. The naturally occurring methylxanthines are caffeine, theophylline and theobromine (Figure 1) (Beale, 2011).

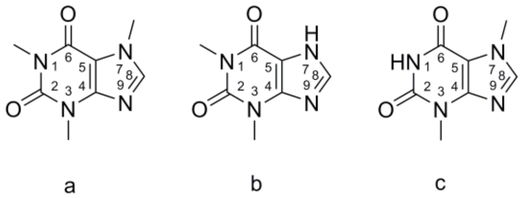

Figure 1. The 2D structures of caffeine (a), theophylline (b) and theobromine (c)

Caffeine is a widely used CNS stimulant. It acts as a cortical stimulant and facilitates clear thinking and wakefulness, promotes an ability to concentrate on the task at hand and lessens fatigue (Nehlig, Daval, Debry, 1992; Fredholm, Battig, Holmen, et al., 1999).

Theophylline has some medical use as a CNS stimulant, but its main use is in therapy for respiratory diseases such as chronic obstructive pulmonary disease (COPD) and asthma, and infrequently as a diuretic (Barnes, 2006; Beale, 2011).

Theobromine has very little CNS activity, similar to caffeine and theophylline but on a much lower scale (probably because of poor physicochemical properties necessary for distribution in the CNS).

Short review of relative pharmacological potencies of the xanthines is summarized in Table 1 (Beale, 2011).

Table 1. Relative pharmacological potencies of the xanthines

Xanthine	**CNS Stimulation**	**Respiratory Stimulation**	**Diuresis**	**Coronary Dilatation**	**Cardiac Stimulation**	**Skeletal Muscle Stimulation**
Caffeine	1^a	1	3	3	3	1
Theophylline	2	2	1	1	1	2
Theobromine	3	3	2	2	2	3

^{a}1, most potent

The CNS-stimulating effects of the methylxanthines were once attributed to their phosphodiesterase-inhibiting ability. This action is probably irrelevant at therapeutic doses. Evidence indicates that the overall CNS-stimulant action is related more to the ability of these compounds to antagonize adenosine at A_1 and A_{2A} receptors (Fredholm, Battig, Holmen, et al., 1999; Daly, 1982; Williams, Huff, 1983; Snyder, Katims, Annau, et al., 1981).

Caffeine and theophylline have pharmaceutically important chemical properties. Both are weak Brønsted bases. Basicity is the result of the imino nitrogen at position 9. Theophylline lacks methyl group at position 7 compared to caffeine, having instead a proton that can be donated. Caffeine cannot donate a proton from position 7. Caffeine does have electrophilic sites at positions 1, 3 and 7. In addition to its Brønsted acid site at 7, theophylline has electrophilic sites at 1 and 3. In condensed terms, both compounds are electron-pair donors, but only theophylline is a proton donor in most pharmaceutical systems (Beale, 2011).

Theobromine differs from caffeine by only one methyl group. However, that single methyl group confers such different properties to caffeine. As a CNS stimulant and diuretic, theobromine is notably less active than caffeine (Pavia, 1973).

Among quantum chemical methods for evaluation of pharmacological effects, DFT has shown significant promise and appears to be adequate for pointing out the changes in electronic structure responsible for pharmacological action (Špirtović-Halilović, Salihović, Džudžević-Čančar, et al., 2013; Stanchev, Mitkov, Georgieva, et al., 2013).

EXPERIMENTAL

Density function theory (DFT) study

In order to explore the theoretical-experimental consistency, quantum chemical calculations were performed with complete geometry optimizations using standard Spartan 10 software. Geometry optimization was carried at B3LYP/6-31G* level of theory. The chemical reactivity descriptors calculated using DFT are: total energy (E), chemical hardness (η), electronic chemical potential (μ) and electrophilicity (ω).

RESULTS AND DISCUSSION

Structural and electronic properties

DFT calculations were performed for caffeine, theobromine and theophylline. Optimized molecular structures of the most stable form are shown in Figure 2.

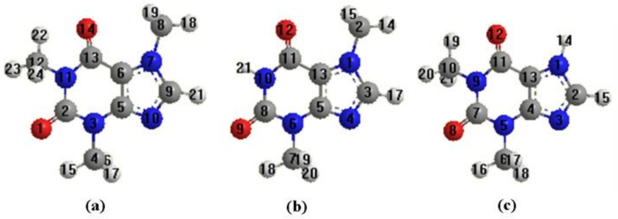

Figure 2. The 3D structures of caffeine (a), theobromine (b) and theophylline (c)

Molecular orbital calculations provide a detailed description of orbitals including spatial characteristics, nodal patterns and individual atom contributions. The contour plots of the frontier orbitals for the ground state are shown in Figure 3, including the Highest Occupied Molecular Orbital (HOMO) and Lowest Unoccupied Molecular Orbital (LUMO).

It is interesting to see that both orbitals are substantially distributed over the conjugation plane. It can be seen from the Figure 3 that the HOMO orbitals are located on the substituted molecule while LUMO orbitals resemble those obtained for the unsubstituted molecule and therefore the substitution has an influence on the electron donation ability, but only a small impact on electron acceptance ability. It can be seen that the energy gaps between HOMO and LUMO of caffeine is 5.12 Hartree, for theobromineis 5.13 Hartree and for theophyline is 5.14 Hartree. The lower value of the HOMO and LUMO energy gap explain the eventual charge transfer interaction taking place within the molecules (Kadhum, Al-Amiery, Musa, et al., 2011).

Chemical hardness is associated with the stability and reactivity of a chemical system, it measures the resistance to change in the electron distribution or charge transfer. On the basis of frontier molecular orbitals, chemical hardness corresponds to the gap between the HOMO and LUMO. Chemical hardness is approximated using equation 1 (Peters, Lanzilotta, Lemon, et al., 1998).

$$\eta = (\varepsilon\text{LUMO} - \varepsilon\text{HOMO})/2 \qquad (1)$$

where εLUMO and εHOMO are the LUMO and HOMO energies.

The larger the HOMO–LUMO energy gap, the harder and more stable/less reactive the molecule (Chattaraj, Maiti, 2003; Liu, 2005; Kadhum, Al-Amiery, Shikara, et al., 2011).

Electronic chemical potential is defined as the negative of electronegativity of a molecule and calculated using equation 2.

$$\mu = (\varepsilon\text{HOMO} + \varepsilon\text{LUMO})/2 \qquad (2)$$

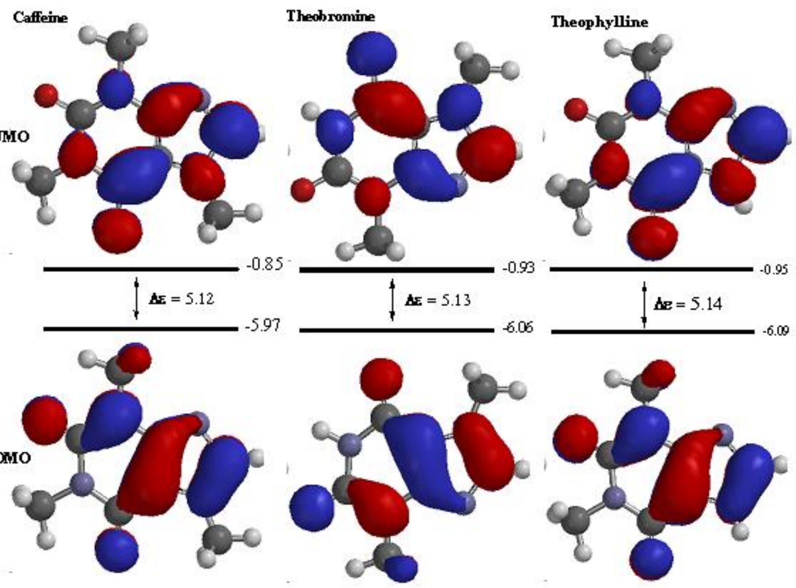

Figure 3. Frontier molecular orbitals of caffeine, theobromine and theophylline

Physically, μ describes the escaping tendency of electrons from an equilibrium system.

Global electrophilicity index (ω), is calculated using the electronic chemical potential and chemical hardness as shown in equation 3.

$$\omega = \mu^2/2\eta \qquad (3)$$

This index measures the propensity or capacity of a species to accept electrons. It is a measure of the stabilization in energy after a system accepts additional amount of electronic charge from the environment (Špirtović-Halilović, Salihović, Džudžević-Čančar, et al., 2013).

Table 2 (row 8) contains the computed chemical hardness values for caffeine, theobromine and theophylline. The results indicate that theophylline is harder and less reactive than theobromine which is harder and less reactive than caffeine. The values of μ for caffeine, theobromine and theophylline are presented in Table 2 (row 7). The trend in electronic chemical potential for methyxanthines is caffeine > theobromine > theophylline.

Table 2. Global chemical reactivity indices of caffeine, theobromine and theophylline

	Caffeine	**Theobromine**	**Theophylline**
E (au)	-680.3764	-641.0661	-641.0621
HOMO (eV)	-5.97	-6.06	-6.09
LUMO (eV)	-0.85	-0.93	-0.95
Dipole moment (debye)	3.80	4.30	3.51
logP	0.17	-0.06	-0.06
Energy gap (Δ) (eV)	5.12	5.13	5.14
μ (eV)	-3.41	-3.49	-3.52
η (eV)	2.560	2.565	2.570
ω (eV)	2.271	2.374	2.410

The greater the electronic chemical potential, the less stable or more reactive is the compound. Therefore, caffeine is the more reactive than theobromine and theophylline. Theophylline is the least reactive. The electrophilicity values (Table 2, row 9) for the caffeine, theobromine and theophylline are 2.271 eV, 2.374 eV and 2.410 eV. Among the compounds, caffeine is the strongest nucleophile while theophylline is the strongest electrophile.

Electrostatic potential charges and related quantum chemical properties

The distribution of the electronic density (electrostatic potential charges), related quantum chemical parameters [dipole moment (Table 2, row 4), HOMO/LUMO gap (Table 2, row 6) and the partition coefficients of the compounds (logP; Table 2, row 5)] were calculated for observed methylxanthines. These values and properties are very useful and can be used in order to evaluate chemical properties and possibilities for interaction of methylxanthines with biological macromolecules (receptors, enzymes) as well as their transport through the cell membranes and brain-blood barrier. All these structural, electronic parameters and logP can be used for a building of quantitative structure-activity relationship (QSAR) model, because all of them are closely related to pharmacokinetics (absorption, distribution, metabolism and excretion) and pharmacodynamics (binding with the target proteins) (Stanchev, Mitkov, Georgieva, et al., 2013).

Redox potential can be measured from HOMO/LUMO gap; it is substantial for the metabolism (oxidative reactions) of these compounds involving cytochrome P450 enzymes. The observed HOMO/LUMO gap of 5.12 – 5.14 eV (Table 2) is used to estimate redox potential. The lower negative HOMO energy and the negative values of LUMO energies indicate that these compounds have the general properties of the reductors. This information may be significant for drug metabolism, as the oxidation (and sometimes the reduction) is the main metabolic pathway for many drug molecules.

All three derivatives are polar molecules, judging by their electric dipole moments (Table 2, row 4). The most polar molecule is theobromine. Theobromine differs from caffeine by only one methyl group. However, that single difference confers such different properties. Free nitrogen at position 1 is part of an imide group. This polarity of theobromine hinders passage through blood-brain barrier and explains its notably lesser activity than caffeine as a CNS stimulant and diuretic. Caffeine is strictly basic compound, while theobromine behaves both as an acid and a base depending on conditions (imide and imino groups).

Caffeine is found to be the most lipophilic according to the logP values (Table 2, row 5). Difference in the substituent at the position 7 between caffeine and theophylline gives caffeine this advantage, thus reputedly achieves higher brain concentrations. The half-life of caffeine is 5 to 8 hours, and that of theophylline, about 3.5 hours (Beale, 2011).

Atomic charges for caffeine, theobromine and theophylline

These datas of caffeine, theobromine and theopylline show that the atomic charges has been affected by the presence of substituent of rings (Peters, Lanzilotta, Lemon, et al., 1998; Kadhum, Mohamad, Al-Amiery, et al., 2011; Kadhum, Wasmi, Mohamad, et al., 2012) as shown in the Tables 3, 4 and 5.

Table 3. Atomic charges of caffeine

Atom	Atom Type (MM2)	Charge (MM2)	Charge Huckel	Atom	Atom Type (MM2)	Charge (MM2)	Charge Huckel
O (1)	O Carbonyl	0	-0.953	C (13)	C Carbonyl	0	0.305
C (2)	C Carbonyl	0	0.367	O (14)	O Carbonyl	0	-0.865
N (3)	N Enamine	0	0.460	H (15)	H	0	0.034
C (4)	C Alkane	0	-0.064	H (16)	H	0	0.036
C (5)	C Alkane	0	0.161	H (17)	H	0	0.037
C (6)	C Alkane	0	-0.063	H (18)	H	0	0.037
N (7)	N Enamine	0	0.441	H (19)	H	0	0.040
C (8)	C Alkane	0	-0.065	H (20)	H	0	0.040
C (9)	C Alkane	0	0.053	H (21)	H	0	-0.000
N (10)	N Imine	0	-0.458	H (22)	H	0	0.033
N (11)	N Amide	0	0.398	H (23)	H	0	0.035
C (12)	C Alkane	0	-0.046	H (24)	H	0	0.036

Table 4. Atomic charges of theobromine

Atom	Atom Type (MM2)	Charge (MM2)	Charge Huckel	Atom	Atom Type (MM2)	Charge (MM2)	Charge Huckel
O (1)	O Carbonyl	0	-0.950	O (13)	C Carbonyl	0	-0.852
C (2)	C Carbonyl	0	0.384	H (14)	H	0	0.034
N (3)	N Enamine	0	0.457	H (15)	H	0	0.036
C (4)	C Alkane	0	-0.064	H (16)	H	0	0.037
C (5)	C Alkane	0	0.169	H (17)	H	0	0.037
C (6)	C Alkane	0	-0.064	H (18)	H	0	0.041
N (7)	N Enamine	0	0.445	H (19)	H	0	0.041
C (8)	C Alkane	0	-0.065	H (20)	H	0	-0.000
C (9)	C Alkane	0	0.056	H (21)	H	0	0.100
N (10)	N Imine	0	-0.457				
N (11)	N Amide	0	0.285				
C (12)	C Carbonyl	0	0.332				

Tabela 5. Atomic charges of theophylline

Atom	Atom Type (MM2)	Charge (MM2)	Charge Huckel	Atom	Atom Type (MM2)	Charge (MM2)	Charge Huckel
N (1)	N Enamine	0	0.331	C (13)	C Alkane	0	-0.042
C (2)	C Alkane	0	0.089	H (14)	H	0	0.085
N (3)	N Imine	0	-0.444	H (15)	H	0	-0.001
C (4)	C Alkane	0	0.168	H (16)	H	0	0.034
N (5)	N Enamine	0	0.463	H (17)	H	0	0.037
C (6)	C Alkane	0	-0.065	H (18)	H	0	0.037
C (7)	C Carbonyl	0	0.366	H (19)	H	0	0.033
O (8)	O Carbonyl	0	-0.953	H (20)	H	0	0.036
N (9)	N Amide	0	0.403	H (21)	H	0	0.035
C (10)	C Alkane	0	-0.046				
C (11)	C Carbonyl	0	0.300				
O (12)	O Carbonyl	0	-0.868				

As a reference compounds, the data for minimized geometry and the 3D-geometrical structures are shown in Figure 3. The Table 3 for caffeine shows that the high estatomic charge in ligand molecule is [O(1) -0.953], next charge value is at [O(14) -0.865] and [N(10) -0.458]. The Table 4 for theobromine shows that the high estatomic charge in ligand molecule is [O(1) -0.950], next charge value is at [O(13) -0.852] and [N(10) -0.457].

The Table 5 for theophylline shows that the high estatomic charge in ligand molecule is [O(8) -0.953], next charge value is at [O(12) -0.868] and [N(3) -0.444]. These data clearly show that these three atoms of caffeine, theobromine and theophylline are the most reactive towards substitution reactions. Angles determined by the bond and the twist angles and the 3D geometric structure indicates that these molecules are planar.

CONCLUSIONS

Caffeine, theophylline and theobromine were investigated, using the hybrid DFT method at B3LYP/6-31G* level of theory. Judging by their electric dipole moments all three derivatives are polar molecules. The most polar molecule is theobromine. Caffeine is found to be lipophilic, according to the logP values and therefore can easily diffuse through the cell membranes and blood–brain barrier.

Polarity of molecules and negative charges are vital for interaction with serum and target proteins by van der Waals forces and electrostatic interactions. Redox potential of all compounds is about 5.12 – 5.14 eV. According to the corresponding HOMO and LUMO values the analyzed structures may be considered as reductors. All these parameters are closely related to pharmacokinetics and pharmacodynamics of biologically active methylxanthines and can be used for a building of quantitative structure-activity relationship (QSAR) model in the future.

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Summary / Sažetak

Koristeći kvantno hemijske metode (teorija funkcionala gustine) istraživani su biološki aktivni metilksantini. Svi proračuni su izvedeni na B3LYP/6-31G* teorijskom nivou. Izračunati su elektronski hemijski potencijal, razlika u HOMO/LUMO energiji, dipolni moment, vibraciona frekvencija, elektrofilnost, hemijska tvrdoća i logP. Dobiveni rezultati se dobro slažu sa nekim farmakološkim osobinama.

Izračunata svojstva bi mogla biti korisna za analizu kvantitativnog odnosa strukture i aktivnosti (QSAR) koja će se obaviti u budućnosti.

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Original Scientific Paper

Phenolic Compounds and Antioxidant Activity of Cocoa and Chocolate Products

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Abstract: Cocoa is the fruit of the tree *Theobroma cacao* L., growing in tropical regions of Africa and South America. Prefermented and fried cocoa serves as a basic raw material for the preparation of chocolate food products. Cocoa is extremely rich in antioxidants, which are responsible for the overall health of the humans. These antioxidants include polyphenols and catechins. This paper presents the investigation of total phenolic contents and antioxidant activity of extracts obtained from cocoa powder and different types of chocolate. The total content of phenolic compounds was determined by spectrophotometric Folin-Ciocalteu method and it varied from 0.046 ± 0.013 to 0.376 ± 0.022 mg GAE/g. The antioxidant activity of the extracts was tested using total antioxidant capacity method. The IC_{50} value was in the range of 1.968 ± 0.076 mg/ml to 42.200 ± 1.737 mg/ml. Cocoa powder and chocolate with a high content of cocoa contain relatively high amount of total phenolics, as well as high antioxidant capacity.

INTRODUCTION

Plants are natural factories for the production of chemical compounds, many of which are used to promote health and fight diseases and some of them are marketed as food or herbal medicines. Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins (Dai and Mumper, 2010; Cheynier, 2012). It is known that the antioxidant activity of the phenolics is primarily a result of their ability to be donors and hydrogen atoms, such as radicals are removed by the formation of less reactive phenoxyl radicals.

Antioxidants are defined as compounds that can delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. Oxidative stress is an imbalanced state where excessive quantities of reactive oxygen and/or nitrogen species (ROS/RNS, e.g., superoxide anion, hydrogen peroxide, hydroxyl radical, peroxynitrite) overcome endogenous antioxidant capacity, leading to oxidation of a varieties of biomacromolecules, such as enzymes, proteins, DNA and lipids. Oxidative stress is important in the development of chronic degenerative diseases including coronary heart disease, cancer and aging (Ames *et al.*, 1993).

Chocolate/cocoa has been known for its good taste and proposed health effects for centuries. Earlier, chocolate used to be criticized for its fat content and its consumption was a sin rather than a remedy, associated with acne, caries, obesity, high blood pressure, coronary artery disease and diabetes. However, the discovery of biologically active phenolic compounds in cocoa has changed this perception and stimulated research on its effects on ageing, oxidative stress, blood pressure regulation, and atherosclerosis.

Cocoa is the mature fruit of the cacao tree (*Theobroma cacao* L.), growing in tropical regions of Africa and South America. The fruit of the cocoa tree has length of about 20 cm and a weight of about 0.5 kg (Komes, 2008). Raw cocoa beans are one of the most nutritious foods in the world, and protects the body from the impact of free radicals, reduces stress and depression, protect against heart disease and blood vessels, protects against many types of cancer, is an excellent source of iron, regulates blood sugar and cholesterol levels, promotes better memory and concentration, reduces the risk of heart attack, and helps regulate blood pressure (Latif, 2013).

Polyphenols in cocoa beans are found in the pigment cells of the cotyledons. Its polyphenolic composition depends on many factors, such as type, geographical origin and growing conditions, and maturity of cocoa fruit (Katz *et al.*, 2011), as well as fermentation and food processing (Goldoni, 2004; Nazaruddin *et al.*, 2006).

This paper presents the investigation of total phenolic content in the extracts isolated from cocoa powder and different types of chocolate, as well as determination of antioxidant activity of isolated extracts.

EXPERIMENTAL

Isolation

Ten grams of grinded samples (cocoa powder and chocolates) were mixed with solvent and extracted using ultrasonic bath for 30 minutes. Each chocolate sample was subjected for two different extraction procedures, extraction with etanol, with/without initial defatting with *n*-hexane.

Determination of phenolics

Total phenolic content was measured using Folin-Ciocalteu spectrophotometric method (Singleton, and Rossi, 1965), using gallic acid for calibration curve. All tests were performed in triplicates, and results are presented as gallic acid equivalents.

Antioxidant activity

Antioxidant activity of isolated extracts was tested using total antioxidant capacity spectrophotometric method (Prieto *et al.*, 1999). The method is based on the ability of potent antioxidant reduce molybdenum ions. All tests were performed in triplicates, and results are presented as IC_{50} values that indicate the concentration of extracts that reduces the 50% of molybdenum. Catechin was as standard probe.

RESULTS AND DISCUSSION

Isolation of phenolic compounds from cocoa powder and chocolates was performed using ultrasonic extraction. Yields of isolated extracts are presented in Table 1, and they do not show high variation for direct extraction from ethanol, while previously defatted extracts do. The percentage from cocoa was taken from declaration of the product.

Table 1: Yields of isolated extracts

Extraction	Sample	Yield (%)
without initial defatting with *n*-hexane	1a	0.36
	2a	0.23
	3a	0.25
	4a	0.25
	5a	0.33
with initial defatting with *n*-hexane	1b	0.36
	2b	0.34
	3b	0.15
	4b	0.41
	5b	2.10

1 – cocoa powder (100% cocoa), 2 – chocolate powder (55% cocoa); 3 – baking chocolate (43% cocoa); 4 – milk chocolate (29% cocoa); 5 – chocolate bar with creamy filling (35% cocoa).

Results from spectrophotometric determination of total phenolic content in isolated extracts are summarized in Table 2 as mg of gallic acid equivalent per gram of extract, and as content of phenolic compounds in extract. Values are represented as the mean taking into account the standard deviation.

Table 2: The phenolic content of extracts of cocoa and chocolates

Sample	Total Phenolics	
	GAE mg/g	(%)
1a	0.376 ± 0.022	10.46 ± 0.60
2a	0.147 ± 0.032	6.74 ± 0.39
3a	0.135 ± 0.006	5.89 ± 0.27
4a	0.097 ± 0.026	3.30 ± 0.10
5a	0.041 ± 0.013	2.05 ± 0.13
1b	0.184 ± 0.014	5.12 ± 0.38
2b	0.051 ± 0.017	3.62 ± 0.27
3b	0.070 ± 0.005	1.85 ± 0.07
4b	0.064 ± 0.009	0.99 ± 0.00
5b	0.046 ± 0.013	0.34 ± 0.03

Total content of phenolic compounds vary from 0.376 ± 0.022 to 0.041 ± 0.013 mg GAE/g, and from 0.184 ± 0.014 to 0.046 ± 0.013 mg GAE/g for non-defatted and defatted extracts, respectively. Presented results are in agreement with those found in the literature (Waterhouse *et al.*, 1996; Ortega *et al.*, 2008; Miller *et al.*, 2009).

Comparing the results obtained for the hexane samples with previously published data by Martos *et al.* (2011), who also used the Folin-Ciocalteu method, it was found that their values are slightly higher. Despite the fact that the samples were defatted before determining polyphenols, cocoa butter interaction with other components of chocolate, during the production of chocolate, may contribute to a different final result, i.e. to increase in the total phenolic content (Jolic *et al.*, 2011). The main limitation of the Folin Ciocalteu method is the lack of specificity, due to interference of other oxidation products during the reaction, and causing variation of the true content of polyphenols (Ainsworth & Gillespie, 2007).

Results of determination of total antioxidant activity using molybdenum reduction method are shown in Table 3, where the IC_{50} present the concentration of extract to reduce 50% of molybdenum cation. Values are represented as the mean taking into account the standard deviation.

The IC_{50} value is in the range of 1.968 ± 0.076 mg/ml to 42.200 ± 1.737 mg/ml that can be compared with antioxidant activity of catechin (2.171 ± 0.023 mg/ml) which was used as standard probe. In general, previously defatted extracts revealed higher antioxidant activity than extracts without initial defatting.

To the best of our knowledge, there are no previously published data concerning evaluation of antioxidant activity of cocoa products using this method.

Table 3: Antioxidant activity of extracts of Cocoa and Chocolate

Sample	**IC_{50} (mg/ml)**
1a	2.201 ± 0.021
2a	2.537 ± 0.233
3a	1.968 ± 0.076
4a	2.040 ± 0.150
5a	3.280 ± 0.309
1b	3.112 ± 0.050
2b	2.244 ± 0.038
3b	4.322 ± 0.051
4b	5.145 ± 0.218
5b	42.200 ± 1.737
Catechin	2.171 ± 0.023

However, even with different method used, presented results are comparable with those found in the literature. Kroyer and Molnar (2011) evaluated antioxidant activity of cocoa and chocolate products using DPPH radical scavenging activity method. They recorded the highest antioxidant activity for cocoa powder, following a dark chocolate with 85% cocoa content.

It seems that the antioxidant activity is correlated with the content of cocoa that is correlated with the content of phenolic compounds in investigated product (Miller *et al*., 2009).

This is in agreement with the fact that antioxidant activity is correlated with the content of phenolic compounds (Muselik *et al*., 2007; Lucena *et al*., 2010). Moreover, Serafini *et al*. (2003) and Halliwell (2003) suggested that milk may interfere with the absorption of antioxidants from chocolate *in vivo* and may therefore negate the potential health benefits that can be derived from eating moderate amounts of dark chocolate.

CONCLUSIONS

Interest in the biological activities of cocoa polyphenols is increasing steadily. In fact, the high polyphenol content of cocoa, coupled with its widespread presence in many food items, render this food of particular interest from the nutritional and "pharmacological" viewpoints.

In summary, the results reported here demonstrate the phenolic content and antioxidant activity of different cocoa and chocolate products. These results indicate that total phenolic content as well as antioxidant activity is dependent of the amount of cocoa in investigated products.

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Summary/Sažetak

Kakao je zreli plod drveta kakaovca koji u sebi sadrži sjemenke u nizovima, a raste u tropskim predjelima Afrike i Južne Amerike. Prethodno fermentiran, a zatim ispržen kakao služi kao temeljna sirovina za dobivanje čokolade. Po svom sastavu, kakao je iznimno bogat antioksidansima koji su odgovorni za ukupno zdravlje organizma. Ti antioksidanti uključuju polifenole i katehine. Ovaj rad predstavlja određivanje sadržaja ukupnih fenola i antioksidativne aktivnosti u ekstraktima dobivenih iz kakaa u prahu i različitih vrsta čokolade, koristeći ultrazvučnu ekstrakciju. Ukupan sadržaj fenolskih spojeva određen je spektrofotometrijskom Folin-Ciocalteu metodom i on varira od 0.046 ± 0.013 do 0.376 ± 0.022 mg GAE/g. Antioksidativna aktivnost ekstrakata ispitana je pomoću metode redukcije molibdena. Vrijednost IC_{50} se nalazi u intervalu od 1.968 ± 0.076 mg/ml do 42.200 ± 1.737 mg/ml. Kakao u prahu i čokolade sa visokim procentom kakaa, sadrže visok sadržaj ukupnih fenola i imaju visoku antioksidativnu sposobnost.

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Original scientific article

Investigation of possibility for reducing AISI 303 stainless steel pitting corrosion by microalloying with boron or zirconium

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Abstract: AISI 303 has the highest machinability comparing with all other austenitic stainless steel grades. The good machinability is result of sulphur presence in the steel composition. Sulphur improves machinability but at the same time causes a decrease in the corrosion resistance. The aim of the research was to examine the possibility of reducing the effect of sulfur content on the corrosion behavior of AISI 303 by microalloying with boron or zirconium. The intention is to keep high machinability of this steel grade but make it corrosion resistant. The results show that after microalloying with boron or zirconium the intensity of pitting corrosion of AISI 303 stainless steel can be significantly reduced. The results show that the effect of reducing the intensity of pitting corrosion of AISI 303 stainless steel microalloyed with boron or zirconium is higher at lower concentrations of chlorides. This is confirmed trough comparing the intensity of pitting corrosion of the 303 stainless steel samples microalloyed with boron or zirconium with the samples of 304 stainless steel. The intensity of pitting corrosion 303 stainless steel microalloyed with boron or zirconium is closer to the intensity of pitting corrosion of 304 stainless steel in 1.5% NaCl solution than in a 3% NaCl solution. Results also clearly indicate that the increase of NaCl concentration leads to an increase of pitting corrosion in all tested samples.

INTRODUCTION

Austenitic stainless steels are among the most widely used types of stainless steel. The most commonly used grades are 300 series of alloys according the American Iron and Steel Institute (AISI). Starting from the basic 304 alloy (Fe-19Cr-10Ni), Mo is added to improve resistance to pitting (2-3 wt.% in the case of type 316 and 3-4 wt.% in type 317). Sensitization due to Cr depletion during welding and other heat treatments, and the possible resultant intergranular corrosion, can be avoided through the use of low-carbon grades (304L, 316L, 317L, in which C is limited to 0.03 wt.% max.) or by adding Ti (type 321) or Nb and Ta (type 347) to precipitate C at higher temperatures. The addition of Cr also imparts greater oxidation resistance, whilst Ni improves the ductility and workability of the material at room temperature (F. King, 2009). Stainless steel has important characteristics such as versatility, durability, attractiveness and high mechanical and corrosion resistance (Kikuti et al. 2004).

Grade 303 is the most readily machinable of all austenitic grades of stainless steel. The good machinability of grade 303 is due to the presence of sulphur in the steel composition. Whilst the sulphur improves machining, it also causes a decrease in the corrosion resistance and a slight lowering of the toughness. The effect of sulfur on the corrosion behavior of austenitic stainless steel is manifested through the behavior of the sulfide inclusions due to the low solubility of sulfur in ferrous metals. sites for localized corrosion for almost 100 years, for steels, and for almost 60 years for stainless steels. The effect of an alloying element can be manifested through effects on the passive film, the local solution chemistry, or the interfacial electrochemical kinetics (Donik et al. 2010). The corrosion resistance of type 303 is lower than that for 304 stainless steel. Type 303 is usually compared with type 304 stainless steel because they have nearly the same chemical composition except for the addition of sulfur in type 303. The toughness is still excellent comparing with other austenitic grades. Grade 303 is used in applications that require parts to be heavily machined. These applications include nuts and bolts, screws, gears, aircraft fittings, bushings, shafts, etc. Sulphur additions to the composition act as initiation sites for pitting corrosion. However, corrosion resistance remains good in mild environments. In chloride containing environments over 60°C, 303 stainless steel is subjected to pitting and crevice corrosion. Grade 303 stainless steel is not suitable for use in marine environments (Atlas Specialty Metals, 2003). Stainless steels generally are subjected to pitting corrosion. Pitting corrosion represents an important limitation to the safe and reliable use of many alloys in various industries. Pitting is characterized by more or less local points of attack with considerable depth and normally occurs on free surfaces. Pitting is a very serious type of corrosion damage because of the rapidity of metallic sections perforation. Pitting corrosion is defined as an extremely localized corrosive attack (Bikić, 2013). Simply stated, pitting is the type of localized corrosion that produces pits, that is, sites of corrosive attack that are relatively small compared to the overall exposed surface (Shreir, 1994).

Pitting corrosion of stainless steels is manifested by the rapid growth of current flow after achieving specific values of anode potential after pits formation. Pitting corrosion can be prevented if the anions present in solution hinder the adsorption of chlorides, or push them from the metal surface. Adding other anions in the solution containing chlorides (chromate, nitrate, Sulfide inclusions have been recognized as preferential environmentally-friendly organic compounds…) moves the value of pitting potential in anodic area.

The resistance to pitting corrosion can be enhanced by increasing the content of chromium, molybdenum and nitrogen in the stainless steel composition. In order to reduce the intensity of pitting corrosion stainless steels are mainly alloyed with molybdenum (ASM International, 1992). For research described in this paper in order to reduce the intensity of pitting corrosion 303 stainless steel is microalloyed with boron or zirconium. Boron in stainless steels increases resistance to general corrosion in acidic environments (Guo Tie-ming et al. 2013). The presence of zirconium in stainless steels also increases corrosion resistance (Cheng Wei-Jen, et al. 2013).

EXPERIMENTAL

The aim of the research was to examine the possibility of reducing the effect of sulfur addition on pitting corrosion in 303 stainless steel microalloying with boron or zirconium. The intention is to keep good machinability of 303 stainless steel but try to make it corrosion resistant. Production of 303 stainless steel microalloyed with boron and zirconium was performed in a vacuum induction furnace with capacity of 20 kg at the Metallurgical Institute"Kemal Kapetanović" in Zenica.

Samples of 304 stainless steel were prepared using commercial 304 stainless steel. Investigations were conducted in the corrosion cell according to Standard ASTM G5, on instrument potentiostat/galvanostat, Princeton Applied Research, model 263A-2, with the software PowerCORR® (Standard, ASTM G5-94). Method of cyclic polarisation was used for corrosion investigation. Corrosion tests were performed on AISI 303 and AISI 304 stainless steels (chemical compositions are given in Table 1).

Investigation was performed in 1,5 and 3,0% NaCl solutions. Tests were performed at room temperature, 20±1°C.

Table 1. Chemical composition of the tested stainless steels

Number of sample	Chemical composition (wt.%)									Type to AISI
	C	Si	Mn	P	S	Cr	Ni	B	Zr	
1 and 5	0,03	0,42	0,61	0,021	0,18	18,3	9,4	-	-	303
2 and 6	0,05	0,47	0,66	0,021	0,19	18,5	9,5	0,004	-	303
3 and 7	0,04	0,35	0,75	0,021	0,17	18,8	9,4	-	0,016	303
4 and 8	≤0,07	≤1,0	≤2,0	max 0,045	≤0,015	17,5-19,5	8,0-10,5	-	-	304

RESULTS AND DISCUSSION

To test pitting corrosion, samples of stainless steel the method of cyclic polarization were used. The method of cyclic polarization includes scanning of the potential to a vertex potential and reverses at the current threshold after crossing the vertex potential. Scan rate was 0.5 mV/s. Pitting corrosion of stainless steels is manifested by the rapid growth of current at achieving specific values of anode potential, pitting potential (E_{pitt}), Figure 1. Pitting potential is the potential at which pitting starts and that phenomenon is noticed when the current of the polarization curve suddenly starts to rise. According to the present understanding of the pitting corrosion process the Epitt represent critical threshold value, where metastable pits nuclei within the passive state of the stainless steel may be transformed into stable growing pits when the passive formation breaks down (Loto et al. 2013).

Negative values of pitting potential mean that steel is prone to pitting corrosion. Surface of hysteresis loop is generally measure of the intensity of pitting corrosion, Figure 1. Larger surface loop means higher intensity of pitting corrosion (Bikić, 2010). The results of pitting corrosion tests of investigated stainless steels are given in Figures 2 to 7.

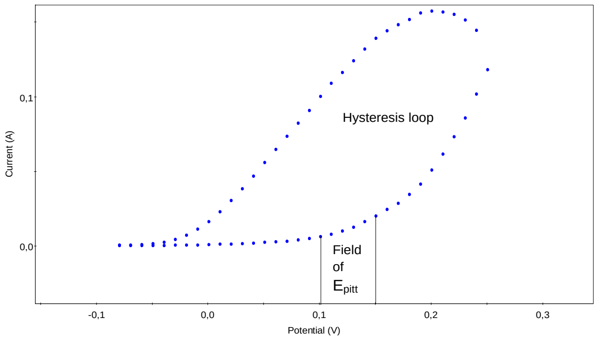

Figure 1. Cyclic polarization curve

Samples 1, 2, 3 and 4 (Table 1) were tested in a 3% NaCl solution. Samples 5, 6, 7 and 8 (Table 1) were tested in a 1,5% NaCl solution. Figures 2 and 3 show the effect of the concentration of chlorides, added over NaCl, on intensity of pitting corrosion of tested stainless steels samples.

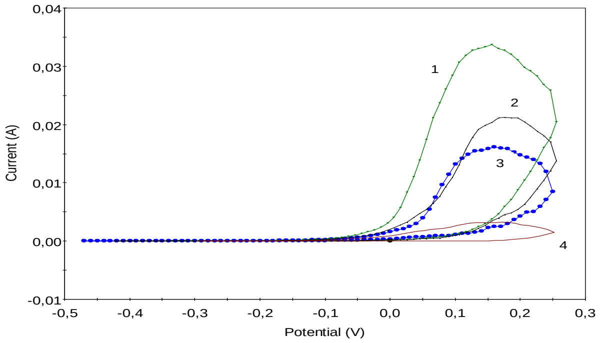

Figure 2. Cyclic polarization curves of samples tested in 3% NaCl solution

Figures 2 and 3 show that, after microalloying of 303 stainless steel with boron or zirconium, the intensity of pitting corrosion is reduced significantly. Comparing the cyclic polarization curves of samples 1, 2, and 3 (Figure 2), as well as samples 5, 6 and 7 (Figure 3) decrease in the surfaces of hysteresis loop of the samples 2 and 3 in relation to surface of hysteresis loop of the sample 1 (Figure 2) as well as the samples 6 and 7 in relation to the sample 5 (Figure 3) is evident. Reducing the surface of hysteresis loop of 303 stainless steel samples microalloyed with boron or zirconium compared to 303 stainless steel that is not microalloyed with mentioned elements, is much more pronounced at lower concentrations of chloride (Figure 3) than at higher concentrations of chloride (Figure 2). This is confirmed by the results of comparing the intensity of pitting corrosion samples of 303 stainless steel microalloying with boron or zirconium with samples of 304 stainless steel.

The intensity of pitting corrosion of 303 stainless steel microalloyed with boron or zirconium is closer to the intensity of pitting corrosion of 304 stainless steel in 1.5% NaCl solution than in a 3% NaCl solution. Namely, it is much better matching of surfaces area of hysteresis loop of the samples 6, 7 to the samples 8 (Figure 3) than surfaces area of hysteresis loop of the samples 2, 3 to the samples 4 (Figure 2).

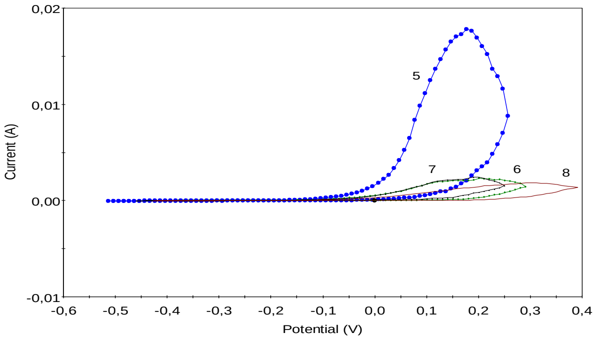

Figure 3. Cyclic polarization curves of samples tested in 1,5% NaCl solution

In general, it can be concluded that 303 stainless steel microalloyed with boron or zirconium becomes much more resistant to pitting corrosion which significantly reduces the effect of sulfur on pitting corrosion. Results also clearly indicate that the increase of NaCl concentration leads to an increase in pitting corrosion in all samples tested. Proof of the above statement is a comparison of pitting potential of samples with same chemical composition treated in solutions with different concentrations of NaCl (Table 2).

Table 2. Values of pitting potential of the tested samples

Number of sample	Conc. of NaCl solution(wt.%)	Pitting potential, ± 10 mv
1	3	66
2	3	76
3	3	30
4	3	192
5	1,5	77
6	1,5	86
7	1,5	90
8	1,5	232

Comparing samples 1 and 5, 2 and 6, 3 and 7, 4 and 8, (Table 2), it is evident that increasing of concentrations of NaCl solution leads to reduction of pitting potential. Reducing the pitting potential means less resistance to pitting corrosion. Comparison of surfaces area of hysteresis loops of samples with same chemical composition treated in solutions with different concentrations of NaCl also proves above mentioned conclusion (Figures 4, 5, 6 and 7). Figures 4, 5, 6 and 7 shows that increasing NaCl concentration leads to an increase of surface area of hysteresis loop which also means an increase of intensity of pitting corrosion. Figures 4, 5, 6 and 7 also show an increase of pitting potential with decreasing concentrations of NaCl.

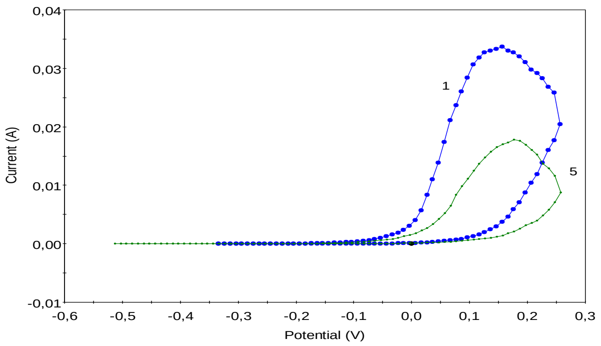

Figure 4. Comparison of cyclic polarization curves

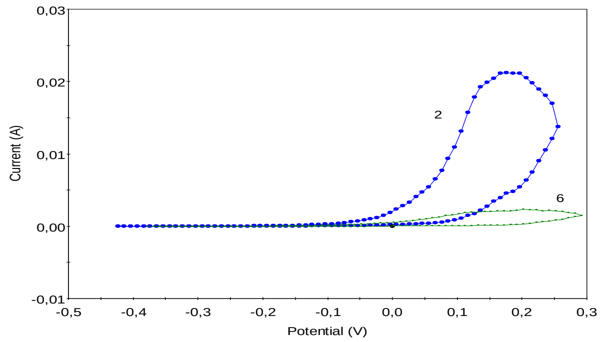

Figure 5. Comparison of cyclic polarization curves

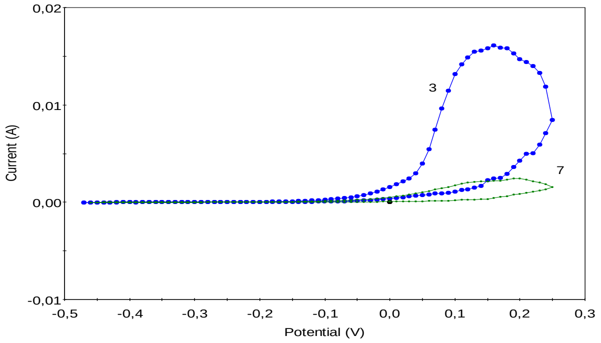

Figure 6. Comparison of cyclic polarization curves

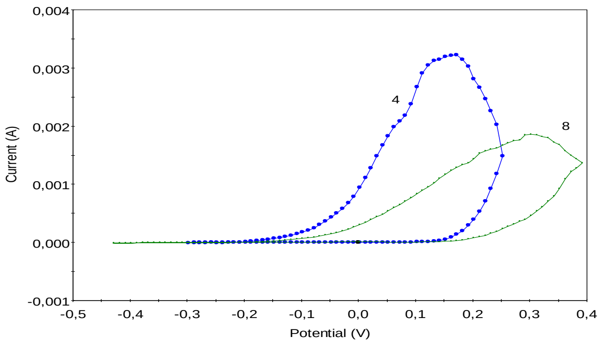

Figure 7. Comparison of cyclic polarization curves

CONCLUSIONS

The results presented in this paper clearly show that the microalloying with boron or zirconium intensity of pitting corrosion of 303 stainless steel can be reduced significantly. This is proven by comparing the intensity of pitting corrosion of 303 stainless steel samples with or without additions of boron or zirconium in solution of 1.5% NaCl and 3% NaCl. The results show that the effect of reducing the intensity of pitting corrosion of 303 stainless steel microalloyed with boron or zirconium is higher at lower concentrations of chloride. This is confirmed trough comparing the intensity of pitting corrosion of 303 stainless steel samples microalloyed with boron or zirconium with 304 stainless steel samples. The intensity of pitting corrosion of 303 stainless steel microalloyed with boron or zirconium is closer to the intensity of pitting corrosion of 304 stainless steel in 1.5% NaCl solution than in a solution of 3% NaCl. Results also clearly indicate that the increase of NaCl concentration leads to an increase of pitting corrosion in all tested samples.

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Summary / Sažetak

Čelik tipa AISI 303 je austenitni nehrđajući čelik koji se najlakše mašinski obrađuje u odnosu na sve druge nehrđajuće čelike zahvaljujući dodatku sumpora. Dodatkom sumpora navedenom čeliku se međutim smanjuje otpornost na koroziju. Cilj provedenih istraživanja je bio ispitati može li se smanjiti efekat dodatka sumpora na povećanje intenziteta piting korozije nehrđajućeg čelika tipa AISI 303, mikrolegiranjem s borom ili cirkonijem. Namjera je zadržati laku obradivost čelika tipa AISI 303 a uz to čelik pokušati učiniti koroziono otpornijim. Rezultati pokazuju da se mikrolegiranjem borom ili cirkonijem intenzitet piting korozije čelika tipa AISI 303 može znatno smanjiti. Rezultati pokazuju da je efekat smanjenja intenziteta piting korozije čelika tipa AISI 303 mikrolegiranjem borom ili cirkonijem veći kod nižih koncentracija hlorida. To potvrđuju i rezultati poređenja intenziteta piting korozije uzoraka čelika tipa AISI 303 mikrolegiranih borom ili cirkonijem s uzorcima čelika tipa AISI 304. Intenzitet piting korozije čelika tipa AISI 303 mikrolegiranog borom ili cirkonijem više se približava intenzitetu piting korozije čelika tipa AISI 304 u otopini 1,5 % NaCl nego u otopini 3 % NaCl. Rezultati takođe nedvojbeno pokazuju da s povećanjem koncentracije NaCl dolazi do povećanja piting korozije na svim testiranim uzorcima.

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Original Scientific Paper

Quantification of some phenolic acids in the leaves of *Melissa officinalis* L. from Turkey and Bosnia

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Abstract: *Melissa officinalis* L. (lemon balm) contains many potentially biologically active compounds, including the caffeic acid (CA), chlorogenic acid (CGA), rosmarinic acid (RA), succinic acid, ursolic acid and thymol. In this study, using the HPLC-ED system, determinations of gallic acid (GA), CGA, RA and CA were performed in hot water extracts of lemon balm. Analyses of GA, CGA, CA and RA were performed in supernatants of lemon balm leaf extracts. The standard solutions of GA, CGA, CA and RA were prepared by dissolving pure substances in the mobile phase. The lemon balm leaves (fresh weight) from Bosnia contained 0.38 mg/g GA, 0.25 mg/g CGA, 0.14 mg/g CA and 5.10 mg/g RA. The lemon balm leaves from Turkey had a higher CA content (0.71 mg/g), while the GA, CGA and RA content was lower (0.22 mg/g, 0.23 mg/g and 0.24 mg/g, respectively). Having many beneficial properties due to the phenolic compounds, lemon should be used as a supplement to a balanced diet.

INTRODUCTION

Melissa officinalis L., commonly known as lemon balm, is a perennial and aromatic herb species of the Lamiaceae family that is native to the Mediterranean region. Due to its ample beneficial properties it is now commercially cultivated worldwide (Shoor, Mondani, Aliverdi et al., 2012). Plants of Lamiaceae family have been used in traditional medicine for treatment of depression, memory enhancement, circulation improvement and indigestion (Shekarchi, Hajimehdipoor, Saeidnia et al., 2012). These plants have shown antioxidant, anti-inflammatory, even anti-carcinogenic properties. Lemon balm is used for several purposes such as an additive in food, a herbal tea, an ingredient in cosmetics, an ornamental and a medicinal plant. It is an aromatic, cooling, sedative herb that lowers fever, improves digestion, relaxes spasms and peripheral blood vessels, and inhibits thyroid activity (Cosge, Ipek and Gurbuz, 2009). In addition, it might present a natural treatment for Alzheimer's disease by amelioration of cognition *(Obulesu and Rao, 2011)*. Other neurological activities include the inhibition of MAO-A and acetylcholinesterase enzymes and affinity to the GABA A-benzodiazepine receptor (Lopez, Martin, Gomez-Serranillos et al., 2009). Most of its medicinal properties the lemon balm owes to a range of different phenolic compounds. Phenolics are characterized by at least one aromatic ring bearing one or several hydroxyl groups. They are mainly synthesized from cinnamic acid, which is formed from phenylalanine (Michalak, 2006). Phenolic compounds are widely distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known. They are ubiquitous in all plant organs and are therefore an integral part of the human diet. Plant phenolics include phenolic acids, flavonoids, tannins and the less common stilbenes and lignans. Phenolic acids can be divided into two classes: derivatives of benzoic acid such as gallic acid (GA), and derivatives of cinnamic acid such as caffeic acid (CA) (Dai and Mumper, 2010). Phenolic acids account for about one third of the total dietary intake of polyphenols. Lemon balm is rich in phenolic acids such as GA, chlorogenic acid (CGA), CA and rosmarinic acid (RA).

Gallic acid is a trihydroxybenzoic acid, often used as a standard for determining the phenol content by the Folin-Ciocalteau assay where results are being expressed in GA equivalents. Having strong antioxidant properties, GA plays a protective role in oxidative stress. It seems to show anti-viral and anti-fungal effects. It has been shown that aqueous extracts from Lamiaceae can drastically and rapidly reduce the infectivity of HIV-1 at non-cytotoxic concentrations (Geuenich, Goffinet, Venzke et al, 2008). Caffeic acid is often esterified with quinic acid as in CGA, which is the major phenolic compound in coffee. Caffeic acid is known to have a broad spectrum of pharmacological activities including anti-inflammatory, antioxidant, immunomodulatory and antiviral (Pari and Karthikesan, 2007). Besides its well-known antioxidant activity, CA inhibits certain enzyme activities such as lipoxygenases, cyclooxygenase, glutathione S-transferase, and xanthine oxidase. It has also been reported to have antitumor activity, anti-inflammatory properties and to inhibit HIV replication (Chung, Moon, Chang et al, 2004). Both, CA and CGA are antioxidants and might inhibit the formation of mutagenic and carcinogenic N-nitroso compounds. They also inhibit the oxidation of LDL and might therefore contribute to the prevention of cardiovascular disease (Olthof, Hollman and Katan, 2001). Results of *in vivo* microdialysis demonstrated the stress relaxing effect of caffeine and CGA on the serotonin and dopamine levels in the rat hippocampus (Pavlica and Gebhardt, 2005). RA, an ester of CA and 3,4-dihydroxyphenyllactic acid was isolated for the first time from *Rosmarinus officinalis* L. Studies suggest that RA is the phenolic acid that is responsible for most of the antioxidant activities of Lamiaceae species extracts. That activity may be even stronger than that of vitamin E or Trolox. The reason could be in the presence of four hydroxyl groups in the molecule of RA. Other phenolic acids have only one or two hydroxyl groups and are not present in sufficient amounts (Caniova and Brandsteterova, 2001). It was also demonstrated that RA is the major compound that drives anxiolytic and antidepressant - like properties of lemon balm (Taiwo, Leite, Lucena et al, 2012). RA has been reported to exert anti-carcinogenic and anti-allergic activities *in vivo* and *in vitro*. Antiviral and antibacterial properties have been demonstrated *in vitro* (Shekarchi et al., 2012).

EXPERIMENTAL

Chemicals

Methanol (HPLC grade) was purchased from Merck, Germany; acetonitrile and glacial acetic acid from Panreac, Spain; the standards for GA, CGA, CA and RA from Sigma Aldrich, Germany.

Chromatographic system

The liquid chromatographic system consisted of a Shimadzu LC-20AT pump (Kyoto, Japan), BAS Liquid Chromatography CC-5E, LC-4C amperometric detector with a glassy carbon working electrode (serial), and a reversed-phase column (ODS Hypersil, 5μm 250 x 4,6 mm, Phenomena). The mobile phase had the following composition: methanol + acetonitrile + water + glacial acetic acid (20+10+70+1). The mobile phase was degassed and filtered through 0.45 μm x 47 mm membrane filters before use. The flow rate was maintained at 1 ml/min. The range detector was 50 nA, the applied potential was + 0.84 V versus the Ag/AgCl reference electrode, the injection volume was 20 μL. The analysis was performed at 25 °C.

Sample preparation

Lemon balm leaves were collected in Bosnia and Herzegovina and Turkey and analyzed for content for the following compounds: GA, CGA, CA and RA. Air dried and powdered herbs (1 g) were extracted with hot water (9 ml) at room temperature for 30 minutes. The plant extract was filtered through a gauze for purification purposes. Afterwards, 1 ml of that extract was centrifuged for 20 minutes, at 15,000 rpm and + 4 ºC (Micro centrifuge, Hettich; Micro 22R). The obtained supernatants were diluted 1/100 with high-purity water. The supernatants were stored at - 20 °C until analysis. A portion (20 µl) of the resulting prepared sample was applied directly to the HPLC system.

The standard solutions for GA, CGA, CA and RA were 0.001 mg/ml, 0.001 mg/ml, 0.003 mg/ml and 0.005 mg/ml, respectively. Their injection volumes were 20 µl.

RESULTS

The quantification was done using LabSolution software of Shimadzu (Kyoto, Japan). Concentrations of GA, CGA, CA and RA were calculated using the equation:

$$\text{Compound}_{(\text{mg/ml})} = \frac{A_{sample}}{A_{s\tan dard}} \cdot \gamma_{(mg/ml)}$$

A_{sample} - peak area for plant sample with the same retention time as standard

$A_{s\tan dard}$ - peak area of the standard

γ - concentration of the standard

The qualitative determination of the listed compounds in samples was based on the comparison of retention times obtained from different extracts of examined plants with the retention time of the corresponding standard. According to the acquired chromatograms, the retention times of GA, CA, CGA, and RA were 3.5 min, 6.9 min, 9.6 min, and 23.6 min, respectively. The list of compounds that were analyzed in plants extracts is shown in Table 1.

Table 1: Content of GA, CGA, CA and RA (mg/g of fresh weight) in lemon balm leaves.

Country /Content (mg/g)	GA	CGA	CA	RA
Bosnia and Herzegovina	0.38	0.25	0.14	5.10
Turkey	0.22	0.23	0.71	0.24

Comparison of phenolic acids content (mg/g of fresh weight) found in lemon balm samples collected in Bosnia and Herzegovina and Turkey is shown in Figure 1. Representative chromatograms of water extracts of lemon balm from Bosnia and Herzegovina and Turkey are shown in Figure 2 and 3.

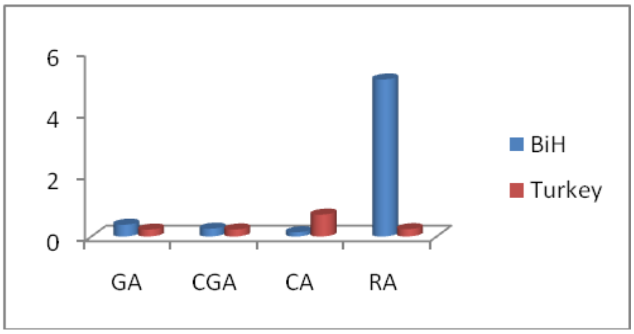

Figure 1: Comparison of phenolic acids content (mg/g of fresh weight) found in lemon balm samples collected in Bosnia and Herzegovina and Turkey.

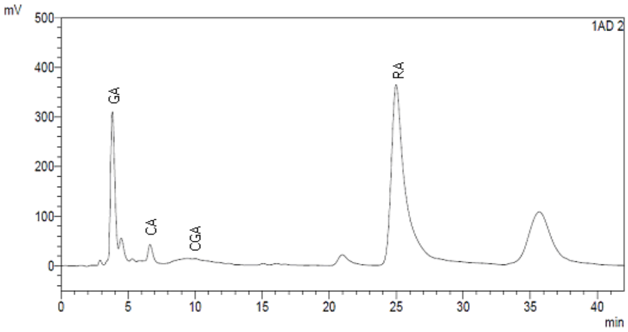

Figure 2: Chromatogram of a lemon balm extract sample from Bosnia and Herzegovina.

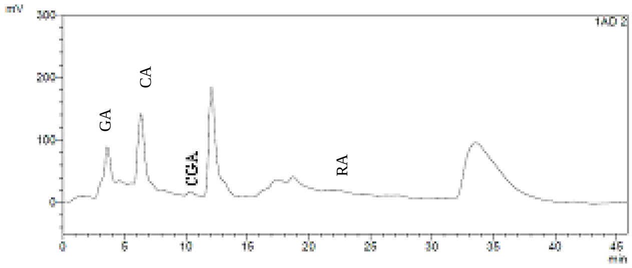

Figure 3: Chromatogram of a lemon balm extract sample from Turkey.

DISCUSSION

Plants are valuable sources of medicinal compounds that contain a broad spectrum of biological activities. Approximately 25 - 50% of current pharmaceuticals are derived from plants and show lesser side effects than the synthetic drugs. The therapeutic properties of lemon balm are mainly ascribed to its content of phenolic compounds. Having antioxidant activities, phenolic compounds show protective effects in terms of cardiovascular, neurodegenerative diseases and even cancer as all these states are linked to oxidative stress. Furthermore, phenolics were found to modulate the activity of a wide range of enzymes and cell receptors. Only recently, the health effects of dietary polyphenols have come to the attention of nutritionists (Dai and Mumper, 2010). Phenolics can be extracted from fresh, frozen or dried plant samples. Usually before extraction plant samples are treated by milling, grinding and homogenization, which may be preceded by air-drying or freeze-drying. It is generally known that the yield of chemical extraction depends on the type of solvents with varying polarities. Methanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols while the higher molecular weight flavanols are better extracted with acetone. Even though extraction temperature can promote the extraction rate, long extraction times and high temperature increase the chance of oxidation of phenolics which decrease the yield of phenolics in the extracts. Specifically, heating tends to hydrolyse RA, which produces CA and results in substantial losses (Caniova and Brandsteterova, 2001). HPLC currently represents the most popular and reliable technique for analysis of phenolic compounds. Acetonitrile and methanol are the most commonly used organic modifiers (Dai and Mumper, 2010). In this study, the mobile phase was acidified with glacial acetic acid to minimize peak tailing. The GA, CGA, CA and RA content in lemon balm samples collected in Bosnia and Herzegovina were 0.38, 0.25, 0.14 and 5.10 mg/g, respectively. The lemon balm leaves from Turkey had a higher CA content (0.71 mg/g), while the GA, CGA and RA content was lower (0.22, 0.23 and 0.24 mg/g, respectively). Overall, plant samples collected in Bosnia and Herzegovina proved to be richer in the phenolic acids content. The lemon balm leaves from Turkey exceeded in the CA content, only. Lemon balm from Bosnia and Herzegovina may exert therapeutic activities more intensively than lemon balm from Turkey. Among the phenolic compounds of interest, RA content was the highest. Being particularly important in preventing oxidative stress and associated diseases, lemon balm with high RA content should be considered a part of regular diet. It should be stressed that comparisons of same plant species from different regions may result in imprecision as the composition of a plant is known to depend considerably on extrinsic and intrinsic factors including soil and climatic conditions, plant ontogenesis phases, harvest and plant storage (Shekarchi et al., 2012). In addition, Rusaczonek, Świderski and Waszkiewicz-Robak (2010) have previously concluded that it would be difficult to compare results obtained by different studies as there are different approaches in extraction procedures, analytical methods and mathematical calculations.

CONCLUSIONS

The obtained results indicate that hot water extracts of lemon balm leaves collected in Turkey and Bosnia and Herzegovina show different contents of phenolic acids analyzed in this study. Overall, samples collected in Bosnia and Herzegovina had a higher content of gallic acid, chlorogenic and rosmarinic acid. In different regions, lemon balm varies in its phenolic content, yet it remains a global natural remedy for infections, indigestion, depression and anxiety, spasms and diseases related to oxidative stress and a source of novel therapeutical tools.

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Summary/Sažetak

Melissa officinalis L. (matičnjak) sadrži mnoge potencijalno biološki aktivne spojeve uključujući kafeinsku kiselinu (CA), hlorogensku (CGA), ružmarinsku (RA), sukcininsku, ursoličnu kiselinu i timol. U ovom radu upotrebom HPLC-ED sistema određivan je sadržaj galne kiseline (GA), CGA, CA i RA u supernatantima ekstrakta listova matičnjaka. Standardne otopine GA, CGA, CA i RA pripremljene su otapanjem čistih supstanci u mobilnoj fazi. Listovi matičnjaka iz Bosne su sadržavali 0.382 mg/g GA, 0.254 mg/g CGA, 0.136 mg/g CA i 5.1 mg/g RA. Listovi matičnjaka iz Turske su imali viši sadržaj CA (0.71 mg/g), dok je sadržaj GA, CGA i RA bio niži (0.22 mg/g, 0.23 mg/g i 0.24 mg/g, respektivno). Sadržaji kiselina su izraženi po masi svježeg lista.

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Original Scientific Paper

Total Phenolic Content and Antioxidant Capacity in Infusions of Various Herbal Teas

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Abstract: Among numerous organic compounds, herbal teas contain various phenolic compounds that may affect the physiological and antioxidant activity of the tea. The aim of this work was to evaluate total phenolic content (TPC) and antioxidant capacity (AC) against peroxide free radicals in the infusions of various herbal teas.
TPC was determined using the spectrophotometric method with Folin-Ciocalteu's reagent, where Me(VI) ions are reduced with phenolics from tea samples to blue-coloured Me(V)-oxides with absorption maximum at 743 nm. Gallic acid, was used as a standard.
AC against peroxyl free radicals, was determined by using the manual spectrofluorimetric method with fluorescein. Trolox (T), which is a hydrosoluble synthetic analogue of natural vitamin E, was used as a standard. TPC varied from 488.8 mg GAE/100 mL (for bearberry tea) to 13.7 mg GAE/100 mL (for sage). Obtained values for AC were in a range 4076.3 μmol TE/100 mL (for bearberry tea) to 251.4 μmol TE/100 mL (for tea against gastritis).

INTRODUCTION

Total phenol content (TPC), and the identification of individual phenolic compounds in plant extracts have been extensively studied since the last decade of the 20th century mainly due to their antioxidant activity (Bors et al., 1984; Cotelle et al., 1996; Zheng and Wang, 2001; Chukarina et al., 2007; Borowska et al., 2009; Orčić et al., 2011), its anti-inflammatory action (Ferrándiz and Alcaraz, 1991), genetic effects (Brown, 1980), and prooxidant action - in the presence of transition metal ions (Ahmad et al., 1992). Today, there are a number of definitions of antioxidants, but none have been generally accepted. Antioxidants are a group of different natural compounds in the human body which are important for protection against the harmful effects of free radicals. There are two sources of antioxidants available. The first refers to the ability of our body to produce antioxidants in the reactions of vitamins and minerals, while second is external source of antioxidants found in various foods (Denisov and Afanas'ev, 2005). It is assumed that the oldest known source of antioxidants is a green tea. The natural world has a lot of antioxidants, and most common are vitamin C and E, phenols and flavonoids.

The aim of this study was to quantify the total phenolic content (TPC) and the antioxidant capacity (AC) of various herbal teas. Twenty samples of herbal teas were analyzed: sage (*Salvia officinalis* L.), green and black tea (*Camellia sinensis* L.), heather (*Calluna vulgaris* L.), bearberry (*Arctostaphylos uva-ursi* L.), peppermint (*Mentha piperita* L.), cranberry (*Vaccinium vitis idaea* L.), mint (*Mentha crispa* L.), chamomile (*Matricaria chamomilla* L.), commercial tea mixture used against gastritis, rosemary (*Rosmarinus officinalis* L.), savory (*Satureja montana* L.), comfrey (*Symphytum officinale*

L.), thyme (*Thymus vulgaris* L.), St. John's wort (*Hypericum perforatum* L.), Turkey malva (*Herniaria glabra* L.), artichoke (*Cynara scolymus* L.), buckwheat (*Fagopyrum esculentum* Moench), tarragon (*Artemisia dracunculus* L.), and a mixture of tarragon and buckwheat. Also, two samples of black tea produced by different manufacturers, and the samples of sage, heather and buckwheat taken from different locations were analyzed.

EXPERIMENTAL

Sample

Twenty five samples of different herbal teas were analyzed for TPC and twenty one for AC.

Sample preparation

One gram of each sample of dried leaves or herb was soaked in 100 ml of boiling, high purity water, left to stand for 10 min, and then filtered into flask of 100 ml. 1.00 ml of this solution was transferred to the flask of 25 ml. Aliquots of 1.5 ml were transferred to centrifugal tubes and centrifuged for 20 minutes at 10 000 rpm at 4 °C. Supernatants were stored in a freezer until analysis.

Standard preparation for total phenolic content (TPC)

Fifty milligrams of the pure gallic acid (GA) was dissolved in distilled water in a 100 mL flask, so that the concentration of GA was 500 mg/L. Stability of this solution is 2 days at 4 °C. This primary standard solution was used for preparation of series of working solutions (10, 25, 50, 75, 100, 150 and 200 mg/L), which in the 0.2 mL aliquots were added in a total volume of 2 ml of the reaction mixture, so that the final concentrations of GA were 1, 2.5, 5, 7.5, 15, and 20 mg/L.

Determination of total phenolic content

The total phenolic content (TPC) was determined using the spectrophotometric method (on PerkinElmer UV/Vis spectrometer Lambda 25) by using Folin-Ciocalteu's reagent, which Me(VI) ions are reduced with phenolics from samples of teas to blue-coloured Me(V)-oxides, with absorption maximum at 743 nm (Singleton and Rossi, 1965; Slinkard and Singleton, 1977). Briefly, 0.2 mL of the supernatant of diluted infusions of tea or working standard was added to 1 ml of 1/10 diluted Folin-Ciocalteu reagent. This mixture was left to stand for 10 minutes. Next, 0.8 ml of 7.5%. Na_2CO_3, was added to the Folin-Ciocalteu reagent. After 30 minutes of incubation at room temperature, the absorbance of solutions was read at 743 nm. TPC was expressed as GA equivalents (mg) per volume of tea infusion (100 ml).

Oxygen Radical Absorbance Capacity (ORAC) Assay

Antioxidant capacity (AC) against peroxyl free radicals, was determined using the manual spectrofluorimetric method with fluorescein as a fluorescent probe. Trolox (T), which is a hydrosoluble synthetic analogue of natural vitamin E, was used as a standard.

Standard preparation for Oxygen Radical Absorbance Capacity Assay

One milligram of trolox was weighed on the analytical balance then dissolved in 0.1 mL of ethanol and supplemented with 3.9 mL of water (initial concentration c_1 = 1 mmol/L). Such solution is stable for 7 days at 4 °C. An aliquot of this solution (40 μL) was supplemented with 1960 mL water giving a solution whose concentration was 20 μmol/L. To prepare the calibration curve, final concentrations of 0.1, 0.25, 0.5, 0.75 and 1 μmol/L of trolox were measured.

Determination of antioxidant capacity

Two analysis of centrifuged sample reacting with peroxide radicals generated from 2,2'-azobis(2-amidino-propane) dihydrochloride (AAPH) were carried out using the Perkin-Elmer luminiscence spectrometer LS 55, with λ_{ex} 485 nm and λ_{em} 520 nm. Fluorescein was used as the target for free radicals attack. The loss of fluorescence of fluorescein is an indication of the extent of damage from its reaction with the peroxyl radical (Cao, and Prior, 1999). Standard was freshly prepared trolox (T), having a final concentration of 1 μmol/L. The total volume of reaction mixture was 2 mL. The mixture contained sample/standard (100 μL), fluorescein (50 μL, final concentration of 8 nM), AAPH (200 μL, final concentration of 64 mM) and water (1650 μL). Mixture containing the sample, fluorescein solution and water was incubated at 37 °C for 15 min. After incubation, AAPH was rapidly added to the mixture to start the reaction. The relative fluorescence intensity (RIF) was measured in batches every 5 minutes after the addition of AAPH. Measurement is repeated until the complete fluorescence quenching (RIF = 0). The samples were termostated at 37 °C during the analysis. The final ORAC values were calculated using a linear equation from calibrated curve. ORAC values were expressed as μmol trolox equivalents (TE) per volume of tea infusion (100 mL).

All measurements were performed in duplicate and results are expressed as mean ± s.d.

RESULTS AND DISCUSSION

Total phenol content (TPC) in herbal tea infusions was determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method using gallic acid (GA) as the standard. Maximum wavelength for blue colored complex was 743 nm. After determination of the λ_{max} of colored complex, the absorbances of seven different concentrations of GA solutions were taken to construct the calibration curve (Keskin-Šašić et al., 2012, Fig. 1).

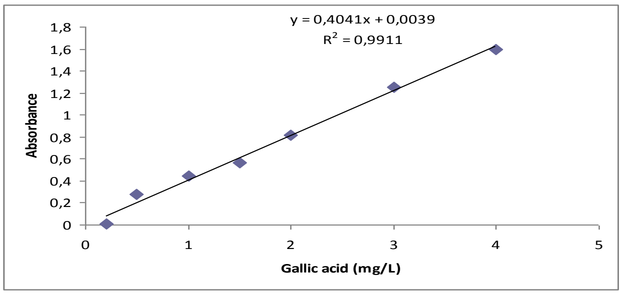

Figure 1: Calibration curve for gallic acid

TPC was determined in 25 different tea samples and mixtures of tea. As shown in Table 1, the values for TPC varied from 488.8 mg GAE/100 mL (bearberry tea) to 13.7 mg GAE/100 mL (sage).
TPC are different for the same tea samples taken from different locations or from different manufacturers. The values were following: sample of black tea I 250.7 mg GAE/100mL, black tea II, 187.1, heather I 79.6, and heather II 25.10, sage I 72.6, sage II 25.11, and sage III 13.7, buckwheat I 231.8, buckwheat II 89.6 mg GAE/100mL.

Table 1: TPC in investigated tea's infusions

Sample	TPC (mg GAE/100mL)
Uva ursi	488.8 ± 28.58
St. John's wort	274.5 ± 7.69
Black tea I	250.7 ± 10.04
Tarragon+Buckwheat mixture	231.8 ± 12.39
Black tea II	187.1 ± 13.90
Tarragon	156.6 ± 44.14
Cranberry	146.3 ± 4.32
Green tea	145.1 ± 15.97
Peppermint	137.1 ± 0.33
Thyme	134.3 ± 6.18
Chamomile	132.9 ± 18.51
Mint	117.2 ± 9.98
Buckwheat I	105.4 ± 13.01
Comfrey	102.4 ± 9.43
Buckwheat II	89.6 ± 19.29
Tea against gastritis	87.9 ± 7.17
Heather I	79.6 ± 11.44
Savory	78.5 ± 23.49
Sage I	72.6 ± 18.37
Artichoke	68.1 ± 6.25
Turkish malva	46.8 ± 6.41
Sage II	25.11 ± 1.65
Heather II	25.10 ± 6.99
Rosemary$_{(Suban)}$	18.9 ± 1.44
Sage III	13.7 ± 2.16

I, II and III: different manufacturer or location

Measurement of antioxidant capacity (AC) was performed by manual ORAC method (Cao and Prior, 1999). Maximum of excitation (λ_{max}=485 nm) and emission (λ_{max}=520 nm) wavelengths were determined using trolox (T) as a standard. After determination of excitation and emission wavelengths, relative fluorescence intensity of five different concentrations of T solutions was used to construct the calibration curve (Fig. 2).

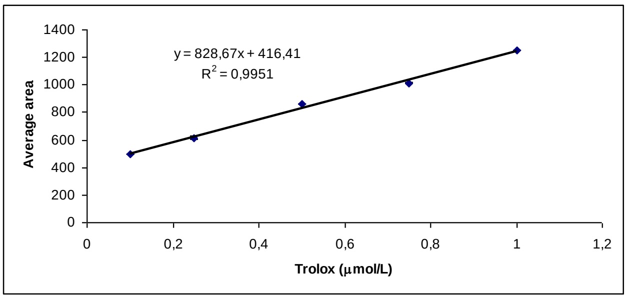

Figure 2: Calibration curve for trolox

The values for AC against peroxyl free radicals in 21 different tea samples are shown in Table 2. As shown in Table 2, there is a big difference in AC values between selected samples. The AC values varied from 4076.3 μmol TE/100 mL (bearberry tea) to 251.4 μmol TE/100 mL (tea mixtures used against gastritis).

Table 2: AC in investigated samples

Sample	AC (μmol TE/100 mL)
Uva ursi	4076.3 ± 227.97
Green tea	3070.9 ± 32.44
Tarragon+Buckwheat mixture	1877.5 ± 209.42
Black tea II	1763.5 ± 123.09
Tarragon	1454.9 ± 77.08
Black tea I	1439.8 ± 141.80
Chamomile	1402.2 ± 26.96
St. John's wort	1366.9 ± 8.21
Comfrey	1328.4 ± 240.17
Peppermint	1322.5 ± 198.53
Artichoke	992.4 ± 41.47
Heather	811.6 ± 48.36
Mint	789.8 ± 205.55
Savory	632.6 ± 223.02
Sage	538.4 ± 7.30
Turkish malva	515.8 ± 86.88
Buckwheat II	359.0 ± 92.60
Buckwheat I	328.2 ± 79.43
Cranberry	323.6 ± 132.04
Thyme	303.6 ± 166.83
Tea against gastritis	251.4 ± 186.13

I, II and III: different manufacturer or location

In summary, although bearberry tea showed the highest values for both analyzed parameters (TPC and AC), the correlation between these values for all tested samples of herbal teas (taking into account bearberry tea) is present only to a certain extent ($r^2 = 0.6303$).

CONCLUSION

The highest TPC was detected in bearberry tea 488.8 mg GAE/100 $mL_{inf.}$, followed by St. John's wort 274.5, black tea I 250.7, tarragon + buckwheat mixture 231.8, black tea II 187.1, tarragon 156.6, cranberry 146.3, green tea 145.1, peppermint 137,1, thyme 134.3, chamomile 132.9, mint 117,2, buckwheat I 105.4, comfrey 102.4, buckwheat II 89.6, tea against gastritis 87.9, heather I 79.6, savory 78.5, sage I 72.6, artichoke 68,1, Turkish malva 46.8, sage II 25.11, heather II 25.10, rosemary 18.9 and sage III 13.7 mg GAE/100 $mL_{inf.}$.

The TPC for the same types of herbal teas but from different locations and different manufacturer, are different. The possible reasons for these differences could be different ways of tea cultivation and period of harvesting, differenences in agronomic procedures, processing, fermentation time and other processes specific for company's production.

The highest AC values against peroxyl free radicals was in bearberry tea (4076.3 μmol TE/100$ml_{inf.}$), followed by green tea 3070.9, tarragon + buckwheat mixture 1877.5, black tea II 1763.5, tarragon 1454.9, black tea I 1439.8, chamomile 1402.2, St. John's wort 1366.9, comfrey 1328.4, peppermint 1322.5, artichoke 992.4, heather 811.6, mint 789.8, savory 632.6, sage 538.4, Turkish malva 515.8, buckwheat II 359.0, buckwheat I 328,2, cranberry 323.6, thyme 303.6, and tea against gastritis 251.4 μmolTE/100$ml_{inf.}$.

Although bearberry tea showed the highest both TPC and AC values, the correlation between these two parameters for all investigated herbal teas (bearberry tea included) was insignificant (r^2=0,6303).

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Summary/Sažetak

Biljni čajevi sadrže brojne organske spojeve, među kojima i različite fenolske spojeve koji mogu uticati na fiziološku i antioksidativnu aktivnost samog čaja. Cilj ovog rada je određivanje sadržaja ukupnih fenola (TPC) i antioksidativnog kapaciteta (AC) protiv peroksidnih slobodnih radikala u infuzama nekih biljnih čajeva. Sadržaj TPC određivan je spektrofotometrijskom metodom koristeći se Folin-Ciocalteu-ovim reagensom, čiji se metalni joni sa fenolima iz uzoraka reduciraju u odgovarajuće plavo obojene okside sa maksimumom apsorpcije na 743 nm, a kao standard korištena je galna kiselina.

AC protiv peroksidnih slobodnih radikala određivan je upotrebom manuelne spektrofluorimetrijske metode sa fluoresceinom, a kao standard korišten je troloks (T), koji je hidrosolubilni sintetski analog prirodnog vitamina E.

Vrijednosti TPC kretale su se u području od 488.8 mg GAE/100 mL (za uvin čaj) do 13.7 mg GAE/100 mL (za čaj od kadulje). Dobijene vrijednosti za AC bile su u području 4076.3 μmol TE/100 mL (za uvin čaj) do 251.4 μmol TE/100 mL (za čaj protiv gastritisa).

DKT KS
DRUŠTVO KEMIČARA I TEHNOLOGA
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Sarajevo, 21.11.2013.

1. Redovna Skupština Društva kemičara i tehnologa Kantona Sarajevo

će se održati u utorak 17. decembra 2013. godine u

17:00 sati, u hemijskom amfiteatru (amfiteatar Mladen Deželić),

Odsjek za hemiju, PMF

Prijedlog dnevnog reda:

1. Godišnji izvještaj o radu Društva u prethodnoj godini i usvajanje izvještaja

2. Ciljevi za narednu godinu
   2a. Osnivanje odbora, komisija i drugih tijela koja pomažu ostvarivanju ciljeva DKT KS
   2b. Inicijativa za organizaciju Simpozija i izbor organizacionog odbora

3. Razno

Predsjednik Upravnog Odbora
Harun Kurtagić

DKT KS
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DRUŠTVO KEMIČARA I TEHNOLOGA
KANTONA SARAJEVO

Sarajevo, 23.12.2013.

ZAPISNIK

1. Redovne Skupštine Društva kemičara i tehnologa Kantona Sarajevo

koja je održana u utorak 17. decembra 2013. godine u

17:00 sati, u hemijskom amfiteatru (amfiteatar Mladen Deželić),

Odsjek za hemiju, PMF

Prisutni:

1. Mustafa Memić
2. Fehim Korać
3. Tarik Fetahagić
4. Ismet Tahirović
5. Aida Šapčanin
6. Vera Dugandžić
7. Jelena Ostojić
8. Safija Herenda
9. Irma Brakmić
10. Zijad Ćehić
11. Melisa Tvrtković
12. Edina Hodžić
13. Hurija Džudžević-Čančar
14. Dalibor Karačić
15. Sanjin Gutić
16. Emina Ferhatbegović-Opanković
17. Lejla Klepo
18. Enis Mašnić
19. Sabina Gojak-Salimović
20. Alisa Selović
21. Mirela Briga
22. Sabina Žero
23. Đenita Kasapović
24. Harun Kurtagić
25. Indira Kozica
26. Dragan Krešić
27. Mirel Subašić
28. Zlatan Rimpapa

Fehim Korać pozdravio je prisutne i naveo da će on kao urednik Glasnika hemičara i tehnologa BiH da vodi Skupštinu, pošto ga je predsjednik Skupštine Hajrudin Hajdar prethodno ovlastio jer je Hajrudin Hajdar opravdano odsutan. Pročitao je predloženi dnevni red koji glasi:

Prijedlog dnevnog reda:

1.Godišnji izvještaj o radu Društva u prethodnoj godini i usvajanje izvještaja

2.Ciljevi za narednu godinu

2a. Osnivanje odbora, komisija i drugih tijela koja pomažu ostvarivanju ciljeva DKTKS
2b. Inicijativa za organizaciju Simpozija i izbor organizacionog odbora

3.Razno

Predloženi dnevni red ***jednoglasno*** je usvojen.

1.Godišnji izvještaj o radu Društva u prethodnoj godini i usvajanje izvještaja

Fehim Korać je pozvao Predsjednika Upravnog odbora Haruna Kurtagića da podnese godišnji izvještaj Društva.
Harun Kurtagić pozdravio je prisutne i prezentirao godišnji izvještaj. Podsjetio je prisutne o članovima raznih odbora, i naglasio da slijedeći članovi nisu prisutni i to: Sanja Ćavar koja je opravdano odsutna zbog postdoktorskog studija, Reuf Bajrović, Hajrudin Hajdar, Emin Sofić, Borivoj Galić, Sead Hrustanović i Faiza Muštović- Biščević. Upravni odbor je održao šest sjednica u prošloj godini, a rezultat rada je, da je obnovljena registracija, obnovljeno članstvo, revidiran Statut, pokrenuta web stranica.
Blagajnik Sabina Žero podnjela je finansijski izvještaj u kojem je rekla da je od članarina prihod bio 850,00 KM, rashod 368,30 KM i trenutno stanje na računu je 481,70 KM.
Fehim Korać, izvjestio je o radu časopisa „Glasnik hemičara i tehnologa BIH“, da trenutno treba da izađe 41 br. Glasnika. Poslije izvještaja o radu Glasnika stavio je na glasanje Izvještaj Društva za prošlu godinu.

Izvještaj o radu Društva u prethodnoj godini ***jednoglasno*** je usvojen.

2.Ciljevi za narednu godinu

Prof. Korać pročitao je ciljeve za narednu godinu, a to su:

-Izrada pravilnika
- Formiranje odbora i komisija
- Iznalaženje načina za finansiranje
-Članstvo
-Organiziranje najmanje jednog stručno-naučnog skupa

Ovu tačku dnevnog reda stavio je na diskusiju.

U diskusiji su učestvovali, Aida Šapčanin, Dragan Krešić, Mustafa Memić, Harun Kurtagić, Sanjin Gutić, Vera Dugandžić, Dalibor Karačić, Enis Mašnić, Mirela Briga, Hurija Čančar, Tarik Fetahagić.

Zaključeno je da treba što više mladih ljudi da se uključe u Društvo, da se afirmišu novi članovi, da se prijavljujemo na projekte za finansiranje Društva, da se Društvo pobrine o hemičarima koji rade u zdravstvenim institucijama, da se izradi udžbenik po IUPAC-u, pošto i oni daju svoj doprinos za afirmaciju, da se zaštite tehnolozi i hemičari.

2a. Osnivanje odbora, komisija i drugih tijela koja pomažu ostvarivanju ciljeva DKTKS

Fehim Korać je rekao prisutnima da je zadatak Upravnog odbora da formira Komisije na osnovu postavljenih ciljeva. Dogovoreno je da svi članovi Društva pošalju e-mailom svoje prijedloge Komisija.

2b. Inicijativa za organizaciju Simpozija i izbor organizacionog odbora

Fehim Korać predlaže da se iduće godine organizuje Simpozij međunarodnog karaktera. Svi prisutni su za organiziranje Simpozija. Aida Šapčanin smatra da treba da se pogleda program nekog drugog Simpozija, pa po njemu da se naprave sekcije. Prijedlog Društva je da se što više mladih kolega uključi, da se formira radna grupa, a zaključak je da se nađe jedna osoba kaja će biti Predsjednik organizacionog odbora i koja će voditi Simpozij i cijelu organizaciju sa ostalim članovima.

3.Razno

Predsjednik Upravnog Odbora Harun Kurtagić upoznao je članove sa ostavkama članova Upravnog odbora Reufa Bajrovića i kolegice Sanje Ćavar i smatra da se trebaju naći njihove zamjene.
Umjesto Reufa Bajrovića predložena je Amela Hrbat, a umjesto Sanje Ćavar predložena je Edina Hodžić.

Prijedlog o novim članovima Upravnog odbora ***jednoglasno*** je usvojen.

Skupština završena u 19:00.

Sarajevo, 23.12.2013. godine

Zapisničar:

Safija Herenda

Ovjerivači zapisnika:	Predsjednik Skupštine:
Lejla Klepo	Hajrudin Hajdar
Jelena Ostojić	

INSTRUCTIONS FOR AUTHORS

GENERAL INFORMATION

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina (Glasnik hemičara i tehnologa Bosne i Hercegovine) is a semiannual international journal publishing papers from all fields of chemistry and related disciplines.

Categories of Contributions

1. *Original Scientific Papers* – (about 10 typewritten pages) report original research which has not been published previously, except in a preliminary form. The paper should contain all the necessary information to enable reproducibility of the described work.
2. *Short Communications* – (about 5 typewritten pages) describing work that may be of a preliminary nature but which merits immediate publication.
3. *Notes* – (about 3 typewritten pages) report unpublished results of short, but complete, original research or describe original laboratory techniques.
4. *Reviews* – (about 30 typewritten pages) present a concise and critical survey of a specific research area. Generally, these are prepared by the invitation of the Editor.
5. *Book and Web Site Reviews* – (about 2 typewritten pages).
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The following is the recommended style for analytical and spectral data presentation:

1. ***Melting and boiling points***:
   mp 163–165°C (lit. 166°C)
   mp 180°C dec.
   bp 98°C
   Abbreviations: mp, melting point; bp, boiling point; lit., literature value; dec, decomposition.
2. ***Specific Rotation***:
   $[a]^{23}_{D}$ –222 (*c* 0.35, MeOH).
   Abbreviations: a, specific rotation; D, the sodium D line or wavelength of light used for determination; the superscript number, temperature (°C) at which the determination was made; In parentheses: *c* stands for concentration; the number following *c* is the concentration in grams per 100 mL; followed by the solvent name or formula.

3. ***NMR Spectroscopy***:

   ^{1}H NMR (500 MHz, DMSO-d_6) d 0.85 (s, 3H, CH_3), 1.28–1.65 (m, 8H, 4´CH_2), 4.36–4.55 (m, 2H, H-1 and H-2), 7.41 (d, *J* 8.2 Hz, 1H, ArH), 7.76 (dd, *J* 6.0, 8.2 Hz, 1H, H-1'), 8.09 (br s, 1H, NH).

   ^{13}C NMR (125 MHz, $CDCl_3$) d 12.0, 14.4, 23.7, 26.0, 30.2, 32.5, 40.6 (C-3), 47.4 (C-2'), 79.9, 82.1, 120.0 (C-7), 123.7 (C-5), 126.2 (C-4).

   Abbreviations: d, chemical shift in parts per million (ppm) downfield from the standard; *J*, coupling constant in hertz; multiplicities s, singlet; d, doublet; t, triplet; q, quartet; and br, broadened. Detailed peak assignments should not be made unless these are supported by definitive experiments such as isotopic labelling, DEPT, or two-dimensional NMR experiments.

4. ***IR Spectroscopy***:

   IR (KBr) n 3236, 2957, 2924, 1666, 1528, 1348, 1097, 743 cm^{-1}.

   Abbreviation: n, wavenumber of maximum absorption peaks in reciprocal centimetres.

5. ***Mass Spectrometry***:

   MS *m/z* (relative intensity): 305 (M^+H, 100), 128 (25).

   HRMS–FAB (*m/z*): $[M+H]^+$ calcd for $C_{21}H_{38}N_4O_6$, 442.2791; found, 442.2782.

   Abbreviations: *m/z*, mass-to-charge ratio; M, molecular weight of the molecule itself; M^+, molecular ion; HRMS, high-resolution mass spectrometry; FAB, fast atom bombardment.

6. ***UV–Visible Spectroscopy***:

   UV (CH_3OH) l_{max} (log e) 220 (3.10), 425 nm (3.26).

   Abbreviations: l_{max}, wavelength of maximum absorption in nanometres; e, extinction coefficient.

7. ***Quantitative analysis***:

   Anal. calcd for $C_{17}H_{24}N_2O_3$: C 67.08, H 7.95, N 9.20. Found: C 66.82, H 7.83, N 9.16. All values are given in percentages.

8. ***Enzymes and catalytic proteins relevant data:***

   Papers reporting enzymes and catalytic proteins relevant data should include the identity of the enzymes/proteins, preparation and criteria of purity, assay conditions, methodology, activity, and any other information relevant to judging the reproducibility of the results[1]. For more details check Beilstein Institut/STRENDA (standards for reporting enzymology data) commission Web site (http://www.strenda.org/documents.html).

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[1] For all other data presentation not mentioned above please contact Editor for instructions.

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