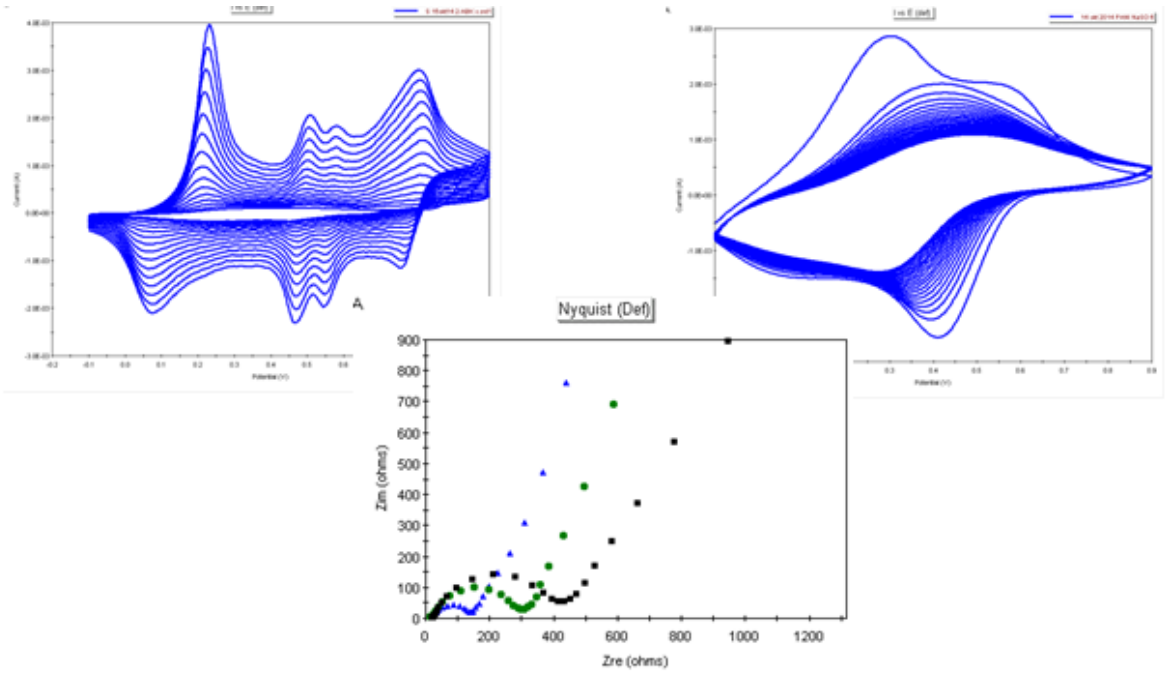
Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

Print ISSN: 0367-4444
Online ISSN: 2232-7266



44

June 2015.

Prirodno-matematički fakultet Sarajevo
Faculty of Science Sarajevo

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

Print ISSN: 0367-4444
Online ISSN: 2232-7266

44

June 2015.

Prirodno-matematički fakultet Sarajevo
Faculty of Science Sarajevo

Glasnik hemičara i tehnologa Bosne i Hercegovine

Print ISSN: 0367-4444
Online ISSN: 2232-7266

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

Zmaja od Bosne 33-35, BA-Sarajevo
Bosnia and Herzegovina
Phone: +387-33-279-918
Fax: +387-33-649-359
E-mail: glasnik@pmf.unsa.ba
glasnikhtbh@gmail.com

REDAKCIJA / EDITORIAL BOARD

Časopis izlazi polugodišnje, a kompletna tekst verzija objavljenih radova je dostupna na http://www.pmf.unsa.ba/hemija/glasnik.

The journal is published semiannual, and full text version of the papers published are available free of cost at http://www.pmf.unsa.ba/hemija/glasnik.

Citiran u *Chemical Abstracts Service*.

Cited by *Chemical Abstracts Service*.

www.cas.org

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

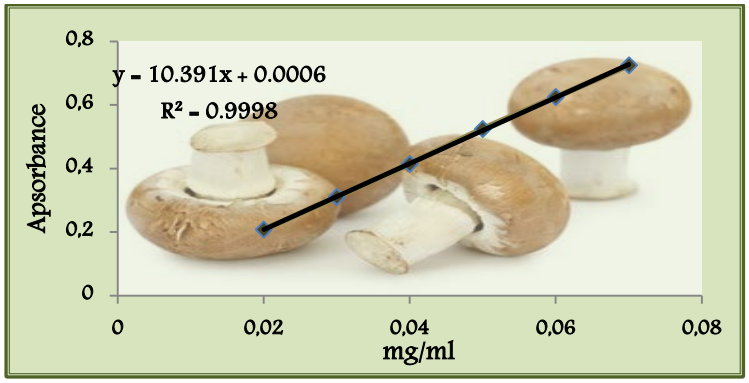
Print ISSN: 0367-4444
Online ISSN: 2232-7266

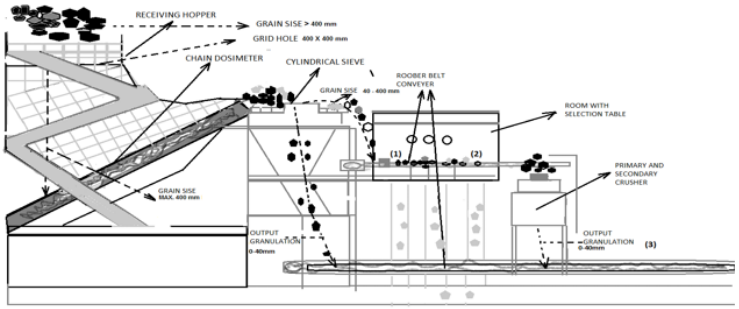
2015
Issue *44*

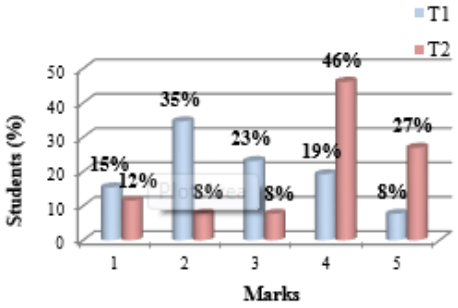
CONTENT / SADRŽAJ

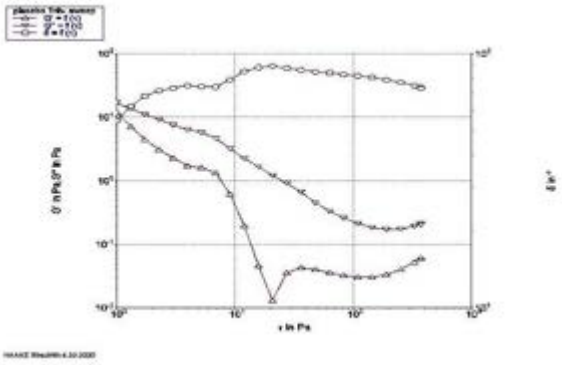
Table 1: Description of analyzed Žilavka white vine samples (vintage 2011).

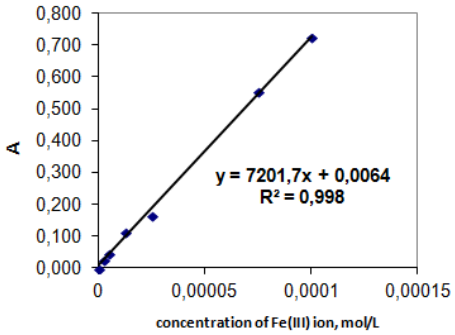
Cod	Winery/Cellar	Location
1	Hepok Ljubuški	Ljubuški
2	Hercegovina produkt	Čitluk
3	V. Sivrić	Međugorje
4	Ostojić	Čitluk
5	Keža	Ljubuški/Studenac
6	Džajo	Ljubuški
7	Buntić	Ljubuški
8	Škegro	Ljubuški
9	Begić *	Ljubuški
10	Begić	Ljubuški

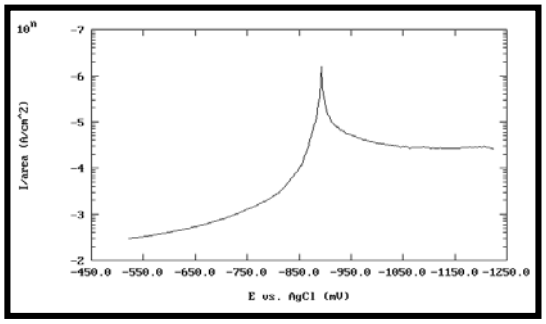


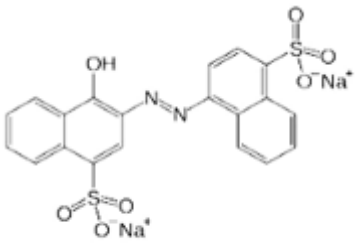


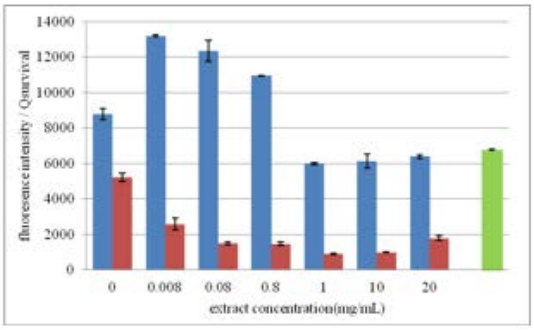


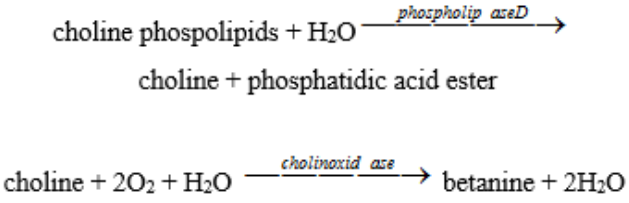


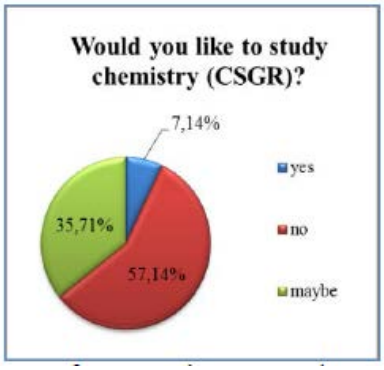












TEHNICAL PAPERS

Water samples	A(^{238}U) (Bq/L)	A(^{234}U) (Bq/L)	Mass(U) (μg/L)	Activity ratio ^{234}U/^{238}U	Chemical yield
1	0.0045	0.0049	0.36	1.09	72%
2	0.0129	0.0175	1.04	1.35	94%
3	0.0112	0.0160	0.90	1.43	98%

Editorial

TEMPUS – NETREL PROJECT: Network for education and training for public environmental laboratories (2012-2015)

NETREL project aims to train researchers and experts in Bosnia and Herzegovina and Serbia in environmental analytical techniques required to meet the major challenges in the monitoring, assessment and management of pollution and emission of toxic compounds in water samples in Western Balkan countries.

The main idea of NETREL is to train public environmental laboratories in Bosnia and Herzegovina and Serbia on local, regional and national level. Also one of the ideas of the NETREL project is to create and implement a harmonized network of universities and public laboratories capable to prepare national and regional strategy for gathering of information on new organic pollutants, building of capacity and establishment of strategic partnerships in order to fill the identified human resources, equipment and data gaps. NETREL action will allow the development and implementation of state-of-the-art methodologies to enable and improve the Western Balkan capabilities for monitoring of environmental pollutants. These will be comparable to those already used in EU member states.

NETREL project consortium consists of 7 universities, 3 EU universities (Slovakia, Czech Republic and United Kingdom) and 4 partner country universities (Bosnia and Herzegovina and Serbia). Seven public laboratories from local, regional and national level in Bosnia and Herzegovina and Serbia have been selected as project participants (Public Health Institute of Canton Sarajevo, B&H, Federal Agromediterranean Institute, Mostar, B&H, Public Health Insitute of Republika Srpska, B&H, Utility company Waterworks Prnjavor, B&H, Serbian Envrionmental Protection Agency, Serbia, Public Health Institute of Vojvodina, Novi Sad, Serbia and Public Utility Company Waterworks and Sewerage Novi Sad, Serbia.

NETREL project coordinator is assoc. Prof. Ivan Spanik from Slovak University of Technology in Bratislava and coordinator for the University of Sarajevo is assoc. Prof. Tidža Muhić-Šarac from Faculty of Science Sarajevo.

Editors

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2015

44

Print ISSN: 0367-4444

Online ISSN: 2232-7266

UDC: _____

Original Scientific Article

1-4

Antioxidant activity and total phenol content of white wine Žilavka

Marković, M.[a*], Martinović Bevanda, A.[b], Talić S.[b]

[a]*Faculty of Agriculture and Food Technology, University of Mostar, Biskupa Čule b.b., 88000 Mostar, B&H*

[b]*Faculty of Sciences and Education, University of Mostar, Matice hrvatske bb, 88 000 Mostar, B&H*

Article info
Received: 12/2/2015
Accepted: 25/5/2015

Keywords:
Antioxidant activity,
phenol content,
white wine, Žilavka,
DPPH reduction,
Briggs-Rauscher oscillating reaction

***Corresponding author:**
Email:markovic_marijana@hotmail.com
Phone: 00-387-63- 465862

Abstract: It is already well known that wine consist of different compounds with strong antioxidant activity. Among them, most common ones are different phenol compounds generally separated in two major groups; flavonoids and nonflavonoids. In this paper we determined total phenol concentration and antioxidant activity of Herzegovinian white wines. Eighteen commercially available white wines made from autochthonous grape varieties Žilavka (vintage 2011) were analyzed. Total phenol content was determined spectrophotometrically according to the Folin-Ciocalteu method using gallic acid as a standard. Two distinct methods were used to assess the antioxidant activity of tested wines: spectrophotometric monitoring of *2,2-diphenyl-1-picryl*hydrazyl (DPPH•) free radical scavenging activity and Briggs-Rauscher (BR) oscillating reaction method.

Total phenol concentration in wine samples varied from 249.3 ±SDmgL^{-1} to 801. ±SDmgL^{-1} expressed as mg of gallic acid equivalent per liter of wine, determined from a standard calibration curve. Similar antioxidant activity was obtained by both performed methods. The antioxidant capacity obtained by DPPH• method ranged from 28.8%±SD to 70.2%±SD. In some cases, the results obtained using both, DPPH• and BR methods, confirmed the fact that wines with higher total phenol content have stronger antioxidant activity.

INTRODUCTION

Antioxidants are synthesized or natural substances that may prevent or delay some types of cell damage by donating electron or hydrogen atom to reactive free radical (Kinsella, Frankel, German et al, 1993), (Pryor, 1991). Antioxidants are found in many foods, including fruits, vegetables and wine. A lot of wine ingredients, including several hundred different phenols, possess strong antioxidant activity and thus are common research topic. The phenolic content in wine refers to large group of chemical compounds that affect the taste, color and taste of wine. These compounds include phenolic acids, stilbenoids, flavonols, dihydroflavonols, anthocyanins, flavanol monomers (catechins) and flavanol polymers (proanthocyanidins) (Kennedy, Matthews and Waterhouse 2012).

Žilavka is native grape variety from the region of Herzegovina. The variety of Žilavka gives quality white wine very often with the addition of 15% of Krkošija and Bena, which are autochthonous varieties of the region of Herzegovina as well.

In the present study, we determined total phenol concentration (according to the Folin-Ciocalteu method) and antioxidant activity using DPPH• (*2,2-diphenyl-1-picryl-hydrazyl*hydrate) radical scavenging and Briggs-Rauscher (BR) oscillating reaction methods in Herzegovinian white wines made from domestic grape Žilavka.

To this date, there has been no research paper on the antioxidant activity and phenol content of white Herzegovinian wine in the literature.

EXPERIMENTAL

Chemicals

All solutions were prepared using analytical-reagent grade substances and Milli-Q deionized water (18.2 MΩ·cm).

Potassium iodate, ethanol, sulphuric acid, hydrogen peroxide, malonic acid, starch, manganese sulfate monohydrate and Folin-Ciocalteau reagent were purchased from Kemika d.d. Zagreb, Croatia. DPPH• reagent and gallic acid were purchased from Fluka Chemie GmbH.

Wine samples

For this study, we selected eighteen controlled geographic origin wines (Table 1), commercially available and widely consumed. All samples were kindly supplied by sixteen private cellars. Wines were stored at room temperature in dark space until analysis. Analysis were performed in June, 2014. All samples were analyzed in triplicates.

Table 1: Description of analyzed Žilavka white vine samples (vintage 2011).

Cod	Winery/Cellar	Location
1	Hepok Ljubuški	Ljubuški
2	Hercegovina produkt	Čitluk
3	V. Sivrić	Međugorje
4	Ostojić	Čitluk
5	Keža	Ljubuški/Studenac
6	Džajo	Ljubuški
7	Buntić	Ljubuški
8	Škegro	Ljubuški
9	Begić *	Ljubuški
10	Begić	Ljubuški
11	Rebac	Čapljina/Trebižat
12	Ereš	Mostar/Sretnice
13	Brkić	Čitluk
14	AG Međugorje	Međugorje
15	Čitluk**	Čitluk
16	Čitluk	Čitluk
17	Zadro	Čapljina
18	Andrija	Čitluk, Paoča

* Vintage 2008;

** Vineyard on special lime stone ground, so-colled *Stone wine*

Apparatus

Shimadzu UV mini-1240 (Shimadzu, Kyoto, Japan) UV-Vis spectrophotometer equipped with a cell (Hellma, Müllheim, Germany) of 10 mm optical path was used. Spectrophotometric data acquisition and control of measurement were achieved by coupling detector with personal computer and using UVmini-1240 data manager software and plug-in memory card with kinetics program both from Shimadzu (Shimadzu, Kyoto, Japan).

Total phenol concentration

Total phenol concentration was determinated spectrophotometricaly by Folin-Ciocalteau method. After adding Folin-Ciocalteau reagents in wine samples, colored product was formed. Folin-Ciocalteau reagent is phosphowolfram and phosphomolybdic acid mixture. During oxidation reaction, phenol groups are oxidized toquinone which are blue colored. Absorption of the resulting solution was measured at 765nm (Amerine and Ough , 1988).

Working solutions were prepared by mixing 0.25 mL of sample (pre-diluted 1:10), 15 mL deionized water and 1.25 mL of Folin-Ciocalteau reagent in 25 mL volumetric flask. One minute after, 3.75mL of sodium carbonate (Na_2CO_3, w=20%) soloution was added and flask was made up to the volume with deionized water. Solutions were stored for two hours in dark space prior the measurements of absorbance at 765nm.

Calibration curve was created using gallic acid as a standard. Seven stock solutions of gallic acid were prepared in the concentration range from 0 to 1000 mg L^{-1}.The volume of 0.25 mL of each solution was trasnfered in 25 mL volumetric flask and treated according total phenols determination method. The results are reported as a gallic acid equivalent γ(GAE) mgL^{-1}.

2,2-diphenyl-1-picrylhydrazyl (DPPH•) antioxidant assay

DPPH• free radical scavenging activity is the basis of a used antioxidant assay. DPPH• free radical method is an antioxidant assay based on electron-transfer reaction that produces a violet solution in methanol (Huang, Ou and Prior, 2005). This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless methanol solution. The use of the DPPH• assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry. Decreas of DPPH• solution absorbance was measured for 30 minutes at 517nm.

For this method DPPH• methanol solution was used (c=6×10^{-5} mol L^{-1}).The volume 1.5 mL of DPPH• solution (absorption≈1) and 25 µL of wine sample was pipetted into the spectrophotometric cuvette. Decreas of absorption was measured for 30 minutes at 517 nm (Yen and Duh, 1994). The percent inhibition (%I) of DPPH• radical by the wine samples was calculated according to equation:

$$\% I = [(A_0-A_t)/A_0]\times 100$$

where A_0 is the absorbance of DPPH• solution without antioxidant at the beginning of measurement (t = 0) and A_t is the absorbance of DPPH• solution containing antioxidant at the end of measurement (t = 30 min).

Briggs-Rauscher (BR) oscillating reaction method

BR oscillating reactions occur as series of reactions which cause color changes in specific time intervals (colorless-yellow-dark blue) (Cervellati, Höner, Furrow et al, 2001). BR oscillating system consists of the iodination and oxidation of an organic substrate (malonic acid) by acidic iodat in the presence of hydrogen peroxide and with the Mn^+ ion as catalyst. The antioxidant leads to immediate cessation of oscillation, and after the so-called inhibition time, the oscillatory behavior is regenerated (De la Rosa, Alvarez-Parilla and Gonzalez-Aguilar, 2010). Duration of reaction cessation is directly affected by type and amount of added antioxidant.

Three solutions were prepared:
1.The iodate solution, 0.2 mol L^{-1},was prepared by dissolving 4.28 g potassium iodate in 0.45 mL concentrated sulfuric acid and diluted with deionized water up to 100 mL.
2. Hydrogen peroxide *w* (H_2O_2)*=15%* (fresh prepared).
3.The mixture with resulting concentration of 0.15 mol L^{-1} for malonic acid, 0.02 molL^{-1} solution of manganese sulfate was prepared by transferring appropriate amount of these substances in 100 mL volumetric flask and dissolving it in 50 mL deionized water. In this mixture, starch solution (0.03% resulting concentration) was also added, and finely diluted up to nominal volume with deionized water. Five milliliters of each of colorless solutions were mixed. Diluted wine samples (1:10) were added to 15 ml of an active, well-stirred (300 r.p.m.) BR mixture, after the third oscillation. Different volumes of diluted wine samples were added: 0.10, 0.25, 0.50 and 0.75 mL, respectively. Inhibition time was measured e.g., till dark-blue color occurs again. Results are expressed graphically as volume and inhibition time linear dependence (Marković and Talić, 2013). The obtained slope of regression line is measure of antioxidant activity; where steeper slope refer to stronger antioxidant activity.

RESULTS AND DISCUSSION

In this paper wide range of phenol concentrations (mean value of 425.23 mgL^{-1}GAE) in selected wine were reported. Measured concentrations of phenolic compounds are presented in Table 2 and results are expressed as γ(GAE) mgL^{-1}. Highest total phenol concentration value was measured in Žilavka produced by Winery Buntić, 801.9 ± 4.0 mgL^{-1} GAE, and the lowest was in Žilavka produced by Winery Škegro 175.0 ± 1.9mgL^{-1} GAE. According to literature (Mitić, Obradović, Grahovac et al, 2010), obtained phenol concentration for Žilavka compared to other researches' results (white wine from Croatia, Greece, Spain, Italy, Czech Republic), who used the same Folin-Ciocalteau methods, are much higher.

Table 2: Phenol content of white wine sample

Cod	Total phenols γ (GAE), mg L^{-1} ±SD
1	376.8 ±2.2
2	623.3 ± 4.1
3	515.6 ± 3.7
4	249.3 ± 2.6
5	310.5 ± 2.0
6	340.6 ± 1.9
7	801.9 ± 4.0
8	175.0 ± 1.9
9	525.9 ± 3.0
10	324.1 ± 2.3
11	395.5 ± 3.1
12	523.9 ±5.0
13	436.4 ± 3.3
14	467.0 ± 1.5
15	419.0 ± 5.1
16	388.3 ±3.8
17	305.2 ± 2.7
18	479.0 ± 2.0

Antioxidant activity was measured by two distinct methods. Results reported by DPPH• method are presented in Table 3. The greatest value of DPPH• radical scavenging, measured at thirtieth minute is in wine number 9; Žilavka produced by Winery Begić (83.1%) while the lowest value is measured in wine number 17; Žilavka produced by Winery Vina Zadro (23.4%). Other researches (Katalinic, 2004) reported slightly weaker antioxidant activity (39.0%–60.2%).
The differences between our values and the published results could be primarily affected by the nature of the analyzed wines, i.e. by their actual contents of phenolic compounds. On the other hand it is difficult to confront our values of antioxidant activity with the literature data, since majority of authors used various methods such as the inhibition of lipid oxidation, DPPH• method with the evaluation of EC50 (the sample concentration necessary to reduce the remaining DPPH• by 50%), and ORAC method (Oxygen Radical Absorbance Capacity) (Stratil, Kubáň and Fojtová, 2008).
Table 3 represent results obtained by Briggs -Rauscher oscillation reactions method. There is a linear relationship between volume of wine added in reaction mixture and time of inhibition. Line slope was created for each sample and compared, higher slope values (steeper slope) represent stronger antioxidant activity (Prenesti, Toso and Berto, 2005).
Greatest antioxidant activity value was measured in wine produced by Winery Buntić (slope=1516.8) which also contain greatest total phenol concentration (801.9 ± 4.0 mg L^{-1} GAE). Weakest antioxidant activity was measured in wine produced by Winery Čitluk (slope = 143.37).

Table 3: Phenol content of white wine sample

Cod	(% I) DPPH*	BR**
1	28.8 ± 0.4	178.37
2	63.0 ± 1.3	443.34
3	47.2 ±1.4	490.61
4	42.8 ± 3.2	231.43
5	31.1 ± 2.0	179.39
6	35.7 ± 0.9	276.12
7	49.2 ± 6.5	1516.80
8	37.4 ± 5.6	393.10
9	83.1 ± 1.6	169.80
10	64.9 ± 5.2	1120.50
11	35.9 ± 3.6	849.50
12	56.2 ± 3.6	1237.10
13	37.3 ± 0.2	793.14
14	37.9 ± 0.1	404.60
15	46.9 ± 1.0	311.02
16	37.1 ± 5.3	143.37
17	23.4 ± 1.4	216.84
18	47.5 ± 1.2	732.65

*Inhibition of DPPH radical ± SD
****** The obtained slope of regression line

No significant correlation between antioxidant activity determinated by DPPH• and BR reaction method was found. This is due to different antioxidant mechanism involved in used methods.
Some literature findings report high correlations for white wine between the used assays (Mitić et al 2010, Katalinić 2004, Hua et al 2009). However, our results are in agreement with those obtained by Fotakis et al. This

research group obtained a weak correlation for white wine as well (Fotakis et al, 2012).
Obviously, there is need for identification of phenols becauce structure and nature of these compounds is also very important for antioxidant activity.

CONCLUSIONS

Samples of white wine Žilavka analyzed in this paper shoved extremely high total phenol concentration and antioxidant activity, compared to other researchers results. The weak correlation between phenol content of all tested wine and antioxidant activity indicate need for further qualitative analysis of phenols and different assays of antioxidant activity.

ACKNOWLEDGMENTS

The research was supported by Federal Ministry of Education and Science of the Federation of Bosnia and Herzegovina, No. 05-14-4297-1/12
and 1000039. The authors thank to the wineries for the supply wine samples.

REFERENCE

Amerine M. A., Ough C. S. (1988*). Methods for Analysis of Musts and Wines*. (1stEd), John Wiley & Sons, New York.

Cervellati R., Höner K., Furrow S., Neddens C. and Costa S. (2001). The Brigs-Rauscher Reaction as a Test of measure the activity of antioxidants. *Helvetica Chimica Acta,84,* 3533–3547.

Fotakis C., Christodouleas D., Zervou M., Papadopoulos K. and Calokerinosa A. C. *(*2012). Classification of wines based on different antioxidant responses to spectrophotometric analytical. *Analytical Letters, 45,* 581–591.

Hua L. *et al*. (2009). Polyphenolic compounds and antioxidant properties of selected China wines. *Food Chemistry, 112,* 454–460.

Huang D. J., Ou B.X., Prior R.L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry, 53,* 1841-1856.

Katalinic V.*et al.* (2004). Antioxidant effectiveness of selected wines in comparison with (+)-catechin. *Food Chemistry,86, 593–600*.

Kinsella, J.E., Frankel, E., German, B. & Kanner, *J* (1993).Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technology,47,*85–89.

Kennedy J.A., Matthews M.A., Waterhouse A.L. (2002). Effect of Maturity and Vine Water Status on Grape Skin and Wine Flavonoids.*American Journal of Enology and Viticulture 53 (4),* 268–274.

Marković M., Talić S.(2013),.Antioksidant Activity of Selected Herzegovinian Wines. *Kemija u industriji, 62(1-2),* 7-12.

Mitić M.N., Obradović M.V., Grahovac Z.B. and Pavlović A.N. (2010).Antioxidant Capacities and Phenolic Levels of Different Varieties of Serbian White Wines. *Molecules*, *15*(3), 2016-2027.

Prenesti E., Toso S., Berto S. (2005). Redox chemistry of red wine. Quantification by an oscillating reaction of the overall antioxidant power as a function of the temperature. *Journal of Agricultural and Food Chemistry,53*, 4220–4227.

Pryor W. A. (1991). The antioxidant nutrients and disease prevention – What do we know and what do we need to find out? *American Journal of Clinical Nutrition, 53,*391–393.

De la Rosa A.L., Alvarez-Parilla E., Gonzalez-Aguilar A.G. (2010). *Fruit and vegetables phytocemicals.* 1stEd), Willey-Blackvel Publishing.

Stratil P., Kubáň V., Fojtová J. (2008). Comparison of the phenolic content and total Antioxidant activity in Wines as Determinated by spectrophotometric method. *Czech Journal of Food Sciences, 26,(5),* 242-253.

Yen G. C., Duh P. D. (1994). Scavenging effect of methanolic extracts of peanut hulls on free radical and active oxygen species. *Journal of Agricultural and Food Chemistry,42,*629–632.

Summary/Sažetak

Brojna istraživanja potvrđuju kako različita vina posjeduju snažan antioksidacijski učinak. Pripisujemo ga fenolnim spojevima koje čine dvije skupine; flavonoidi i neflavonoidi. U ovom radu određivana je koncentracija fenola i antioksidacijski učinak u bijelom vinu sorte Žilavka iz područja Hercegovine. Analizirano je osamnaest komercijalno dostupnih vina - berba 2011. Koncentracija ukupnih fenola određena je spektrofotometrijski pomoću Folin-Ciocalteau reagensa koristeći galnu kiselinu kao standard. Dvije različite metode su korištene za procjenu antioksidacijskog učinka analiziranih vina. Metoda redukcije 2,2-difenil-1-picrilhidrazil (DPPH$^{\bullet}$) slobodnog radikala te metoda Briggs-Rauscher (BR) oscilirajućih reakcija.Dobivene koncentracije ukupnih fenola su u rasponu od 249,3 ±SD mgL^{-1} do 801, ±SD mgL^{-1} galne kiseline po litru vina, što je značajno više od dobivenih vrijednosti drugih istraživača za bijela vina. Slične vrijednosti antioksidacijskog učinka dobivene su prema obje korištene metode. Antioksidacijski učinak prema metodi redukcije slobodnog radikala DPPH$^{\bullet}$ je u rasponu od 28,8%±SD do 70,2%±SD. Rezultati dobiveni za pojedine uzorke vina upućuju kako vina sa većim sadržajem fenola imaju snažniji antioksidacijski učinak.

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2015

44

5-8

Print ISSN: 0367-4444
Online ISSN: 2232-7266

UDC: _____

Original Scientific Article

Phenolic content and antioxidant activity of mushroom extracts from Bosnian market

Alispahić, A., Šapčanin, A.*, Salihović, M., Ramić, E., Dedić, A., Pazalja, M.

Faculty of Pharmacy, University of Sarajevo, Zmaja od Bosne 8 - Kampus, Sarajevo, Bosnia and Herzegovina

Article info
Received: 6/5/2015
Accepted: 25/5/2015

Keywords:
mushrooms, antioxidant activity, monomeric anthocyanine content, total phenolic content

***Corresponding author:**
E-mail: ida@bih.net.ba
Phone: 00-387-33-586-187
Fax: 00-000-00-0000000

Abstract: Mushrooms are well balanced food that provides definite nutrition and health benefits for humans. Mushrooms are known to produce different kinds of bioactive compounds, generally linked with mycelial cell wall, that help in enhancing the capacity of immune system to fight against carcinogens. To consider the importance of polyphenolic compounds and its presence in many varieties of mushrooms, the total antioxidant activity of dry boletus mushroom, white and brown champignon, oyster mushroom and shiitake from bosnian markets was determined. Total phenolic content was estimated as Galic acid equivalents /g spectrophotometrically according to the *Folin-Ciocalteus* method. Total anthocyanine content was analysed by pH differential spectrophotometric method at 525 and 700 nm. The radical scavenging activity (RSA) of mushroom extracts was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The analysis revealed that the total phenolic contents ranged from 4.94 mg $GAEg^{-1}$ in oyster mushroom to 35.56 mg $GAEg^{-1}$ in dry boletus mushroom. DPPH scavenging activity was the highest for brown champignon with value of 88.33 % and the lowest one was for oyster mushrooms with value of 43.88 %. The mushrooms examined in the present study could represent easily accessible sources of natural antioxidants.

INTRODUCTION

Mushrooms have been a part of human diet in many regions of the world for centuries due to organoleptic characteristic as well as the nutritional values (Wang and Xu, 2014). In nature, there are over 150 000 different types of mushrooms but only 10% is known and designated (Wasser, 2010). However, only about 2 000 species are grown and cultivated for nutritional purposes. The consumption of the mushrooms has even increased remarkably over the past few decades (Gan et al., 2013). Mushrooms are tasteful food, full of proteins, rich in vitamin B, rich in different minerals and have almost all essential amino acids (Mujić et al., 2011). Examination of antioxidant activity in mushroom extracts and content of antioxidant compounds is currently very interesting aim of research.. Mushrooms are found to be rich source of these antioxidants with immense antiradical activity (Valentão et al., 2005).

Phenolic acids were the major phenolic compounds reported in mushrooms.

The antioxidant activity of anthocyanins including the protection of low density lipoproteins (LDL) against oxidation, has been demonstrated in a number of different *in vitro* systems. Phenols are important plant constituents because of their scavenging ability due to their hydroxyl groups (Hatano et al., 1989). In this study the radical scavenging activity (RSA) as well as the polyphenolic content and anthocyanins content of five edible mushrooms (*Boletus edulis, Agaricus bisporus, Agaricus bisporus var. Avellaneous, Pleurotus ostreatus, Lentinula edodes*) commercially available at Bosnian market was investigated. The amount of total phenol content was determined by *Folin-Ciocalteu* reagent method and total monomeric anthocyanin content was determined by pH differential spectrophotometric method.

MATERIAL AND METHODS

Plant material

Mushroom samples of boletus mushroom *(Boletus edulis)*, champignon white *(Agaricus bisporus)*, champignon brown *(Agaricus bisporus var. Avellaneus)*, oyster mushroom *(Pleurotus ostreatus)*, shiitake *(Lentinula edodes)* were collected in Bosnian market. Identification was done by comparing their morphological, anatomical and physiological characteristics and monographs with descriptions given in the manual (Moser, 1983; Bessette et al., 2000; Uzelac, 2009). The investigated mushrooms are five of the most commercially cultivated ones available in Bosnia.

Chemicals and reagents

Folin-Ciocalteu reagent (Kemika, Zagreb, Croatia), gallic acid (Fluka Chemica, Switzerland), anhydrous sodium carbonate (Kemika, Zagreb, Croatia) and methanol (Merck, Darmstadt, Germany), 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma Aldrich, St. Louis, USA) and (Trolox) 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxilic acid (Sigma Aldrich, St. Louis, USA), hydrohloric acid (Sigma Aldrich, St. Louis, USA), potassium chloride (Fluka Chemica, Switzerland), acetic acid (Fluka Chemica, Switzerland), sodium acetate (Sigma Aldrich, St. Louis, USA) cyanidine-3-galactoside (Fluka Chemica, Switzerland) were used in this study. All the chemicals were of analytical grade purity.

Sample preparation

The fresh mushrooms 0.5 g were cut into small pieces, crushed in a mortar with pestle and consecutively extracted with 10 mL of 80 % ethanol. After maceration, extracts were put in centrifuge (Tehnica Železniki LC-320) at 4000 rpm for 20 min. and then the supernatant was separated. These obtained extracts were used for further investigations.Obtained extracts were stored in a refrigerator at 4^{o}C until analysis.

Determination of total phenolic content

Total phenols (TP) were determined spectrophotometrically with *Folin-Ciocalteu* reagent (Waterhouse, 2002). The sample (2 mL) was dissolved in ethanol and mixed with 10 mL *Folin-Ciocalteau's* reagent diluted 1/10 with distilled water. After few minutes sodium carbonate (8 mL) was added to this solution. This solution was stored in dark place for two hours and after that, the absorbance was measured at 765 nm. A standard curve was prepared using gallic acid as standard with a concentration range from 100 to 500 μg/mL. Results are expressed in mg of gallic acid equivalents per gram (mg GAE g^{-1}) of mushrooms.

Determination of total monomeric anthocyanins

Total monomeric anthocyanins (TMA) content was quantified using a pH differential method (Giusti and Wrolstad, 2001). Samples were diluted in two buffer solutions: potassium chloride buffer 0.025 M (pH 1.0) and sodium acetate buffer 0.4 M (pH 4.5) and then the absorbance was measured simultaneously at 525 nm and 700 nm, after 15 minutes of incubation at room temperature. Absorbance readings were made at room temperature using distilled water as blank. A Spektronic Genesiys TM2 UV-Vis spectrophotometer was used for determination. The content of total monomeric anthocyanins was expressed in mg of cyanidin-3-glucoside equivalents (CGE) per gram of mushrooms. A molar extinction coefficient of cyanidin-3-glucoside of 26900 Lmol^{-1}cm^{-1} and molar weight (MW) (449.2 gmol^{-1}) were used for calculations. The anthocyanin concentration was calculated according to the following equation:

$$\text{mg CGE/g} = \frac{\text{A x } M_W \text{ x } D_f \text{ x } 1000}{\varepsilon \text{ x } l}$$

where:

$$A = (A_{525} - A_{700})pH_{\ 1.0} - (A_{525} - A_{700})pH_{\ 4.5}$$

M_W- molecular weight of cyaniding-3-glucoside
D_f- dilution factor,
ε - molar absorbance
l - pathlength

DPPH Radical Scavenging Activity Assay

The mushroom extracts were mixed with methanol (96 %) and 63 μmol/L solution of DPPH. After 30 min. at room temperature, the apsorbance was measured at 517 nm and converted into percentage of radical scavenging activity (Zeković et al., 2010). The comparative analysis of samples was made by calculating DPPH scavenging activity which stands for the relative decrease of absorbance in the samples analysed. DPPH scavenging activity was calculated by using the following equation:

$$\text{DPPH } scavenging \text{ activity} = 100 \times (Ac - As)/Ac$$

where:
A_c - absorbance of the control
A_s- absorbance of the sample.

RESULTS AND DISCUSSION

Results obtained for total phenolics, total monomeric anthocyanins and DPPH scavenging activity are presented in Table 1.

Table 1: Total phenolic content, total monomeric anthocyanins content and DPPH scavenging activity of dry boletus, champignon white, champignon brown, oyster and shiitake mushrooms

Name	TP (mg GAE /g)	TMA (mg CGE/g)	%RSA
dry boletus mushroom *(Boletus edulis)*	35.56	-	87.74
champignon white *(Agaricus bisporus)*	6.43	0.134	87.77
champignon brown *(Agaricus bisporus var. Avellaneus)*	7.66	-	88.33
oyster mushroom *(Pleurotus ostreatus)*	6.27	-	43.88
shiitake *(Lentinula edodes)*	4.94	0.134	71.85

TP- Total phenols; TMA - Total monomeric anthocyanins; RSA - Radical Scavenging Activity

It can be seen that total phenolics content ranged from 4.94 mg GAE g^{-1} to 7.66 mg GAE g^{-1} (fresh weight) and 35.56 mg GAE g^{-1} (dry weight). According to review given by Mujić et al. (2011), content of total phenolics in mushrooms obtained in the range 7.8-23.07 mg GAE g^{-1}. 29.49 to 32.21 mg GAE g^{-1} was determined by different investigators (Yildirim et al. 2012). On the other hand, it is difficult to compare our results with finding of other authors due to differences in extraction method applied, mode of expression of results (on dry or fresh basis of mushrooms), etc. For instance, Yildirim et al. (2012) used methanol to extract bioactive compounds from dry mushrooms. Ejelonu et al. (2013) extracted bioactive compounds from dry mushrooms with distilled water and obtained results in the range from 103.34 mg GAE g^{-1} to 123.35 mg GAE g^{-1}. Furthermore, concentration of total phenols in medicinal mushrooms was between 4.45 mg GAE g^{-1} to 14.44 mg GAE g^{-1} (Abugri and McElhenney, 2013). Our results showed that the highest value of 7.66 mg GAE g^{-1} was deteminated for fresh champignon brown and the highest value of 35.56 mg GAE g^{-1} for dry boletus mushroom.

Total monomeric anthocyanins in investigated extracts were detected in champignon white and shiitake in an amount of 0.134 mg CGE g^{-1} of fresh sample. Research resaults related to anthocyanin content in mushrooms are scarces.

Antioxidant activity of mushroom extracts investigated by DPPH method are presented in Table 1. The antioxidant activity of brown champignon extract exhibited a significant inhibition of DPPH with 88.33 %RSA and the lowest value determinated for oyster mushrooms was 43.88 %RSA. Dry boletus, white and brown champignons showed a higher capacity of scavenging the DPPH radical than the extracts of oyster mushrooms and shiitake. These results can be considered as high antioxidant capacity, in the comparison with antioxidant capacity from various extracts of edible mushroom (*Pleurotus eous)* (Sudha et al., 2012).

Correlation between specific classes of bioactive compounds and antioxidant activity was also investigated. Obtained results are presented in Figure 1.

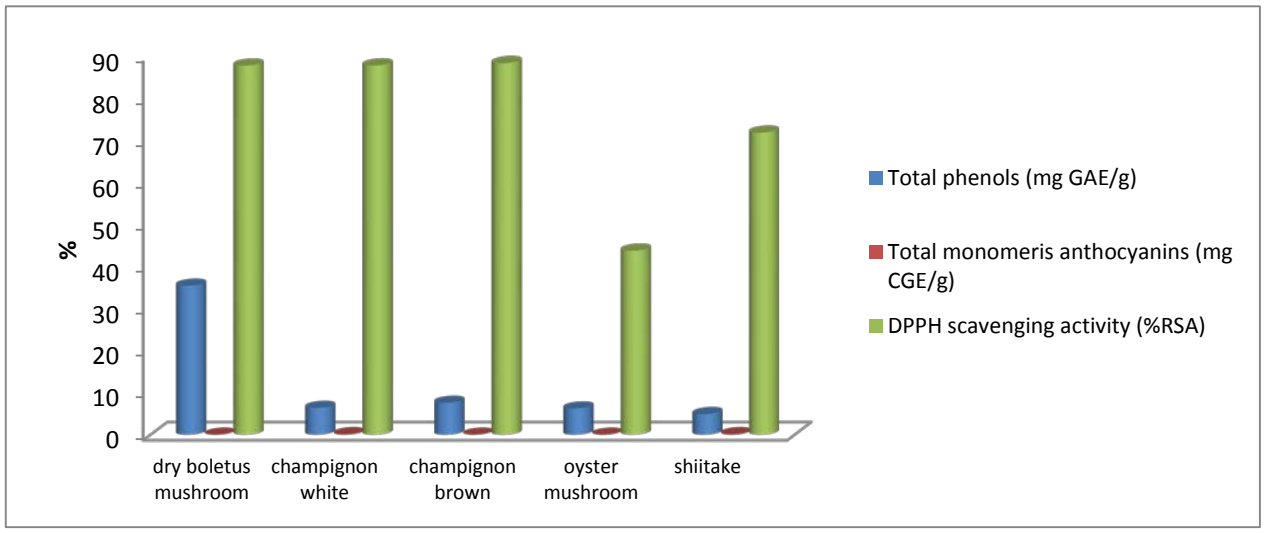


Figure 1. Correlation between DPPH scavenging activity, total phenolic content and total anthocyanin monomeric content

Maximum value of 88.33 %RSA was determined for champignon brown, and minimum value of 43.88 %RSA was determined for oyster mushroom, and was about 50% lower than those of champignons brown, and it was not in correlation with total phenolic content of investigated mushrooms.

CONCLUSIONS

The results of this study indicate that examined mushroom extracts possess good antioxidant activity. In all examined samples phenolic compounds have been detected, but monomeric anthocyanin compounds were detected only in champignon white and shiitake. Due to their high content of antioxidants, extracts of some mushrooms, especially champignon white and boletus, may be used as materials of dietary supplements. Their rich antioxidant contents make the mushroom ideal nutritional supplement. Considering that mushrooms are of yellow, white, brown and dark hue, researchers have found that they are a good source of anthocyanins. Further studies are needed to identify which phenolic compounds are responsible for the antioxidant activity of the species, and assess the way in which the phenolic substances contribute to this activity. Additional *in vivo* antioxidant assays are necessary to confirm the potential use of these species in the treatment of different deseases. So we can conclude that those mushrooms represent a rich source of phenolic compounds and thereby might serve as possible nutraceutical food in human diet, and could help in the reducing the oxidative damage.

REFERENCES

Abugri, D. A., & McElhenney, W. H. (2013). Extraction of total phenolic and flavonoids from edible wild and cultivated medicinal Mushrooms as affected by different solvents. *Journal of Natural Product and Plant Resources*, *3*(3), 37-42.

Bessette, A. E., Roody, W. C., Bessette, A. R. (2000). North American Boletes: A Color Guide to the Fleshy Pored Mushrooms. Syracuse, NY: Syracuse University Press; 1–400.

Ejelonu, O. C., Akinmoladun, A. C., Elekofehinti, O. O., & Olaleye, M. T. (2013). Antioxidant profile of four selected wild edible mushrooms in Nigeria. *Journal of Chemical and Pharmaceutical Research*, *5*(7), 286-295.

Gan, C. H., Nurul Amira, B., & Asmah, R. (2013). Antioxidant analysis of different types of edible mushrooms (Agaricus bisporous and Agaricus brasiliensis). *International Food Research Journal*, *20*(3), 1095-1102.

Giusti, M. M., & Wrolstad, R. E. (2001). Characterization and measurement of anthocyanins by UV-visible spectroscopy. *Current protocols in food analytical chemistry*. John Wiley & Sons, Inc. New York F1.2.1-F1.2.13.

Hatano T., Edamatsu R., Mori A., Fujita Y., Yasuhara E. (1989). Effect of interaction of tannins with co-existing substances. VI. Effects of tannins and related polyphenols on superoxide anion radical and on DPPH radical. *Chemical and Pharmaceutical Bulletin (Tokyo)*. 37. 2016–2021.

Moser, M. (1983). In: Keys to Agarics and Boleti (Polyporales, Boletales, Agaricales, Russulales) Kibby, G, editor. London: Roger Phillips, 1–535.

Mujić, I., Zeković, Z., Lepojević, Ž., Vidović, S., & Živković, J. (2011). Antioxidant properties of selected edible mushroom species. *Journal of Central European Agriculture*, *11*(4). 387-391.

Sudha, G., Vadivukkarasi, S., Shree, R. B. I., & Lakshmanan, P. (2012). Antioxidant activity of various extracts from an edible mushroom Pleurotus eous. *Food Science and Biotechnology*, *21*(3), 661-668.

Uzelac, B. (2009) Gljive Srbije i Zapadnog Balkana. Beograd: BGV Logik, 1–462.

Valentão, P., Lopes, G., Valente, M., Barbosa, P., Andrade, P. B., Silva, B. M., ... & Seabra, R. M. (2005). Quantitation of nine organic acids in wild mushrooms. *Journal of agricultural and food chemistry*, *53*(9), 3626-3630.

Wang, Y., & Xu, B. (2014). Distribution of antioxidant activities and total phenolic contents in acetone, ethanol, water and hot water extracts from 20 edible mushrooms via sequential extraction. *Austin Journal of Nutrition and Food Sciences*, *2*(1), 1-5.

Wasser, S. P. (2010). Medicinal mushroom science: history, current status, future trends, and unsolved problems. *International Journal of Medicinal Mushrooms*, *12*(1). 1-16.

Waterhouse, A. L. (2002). Determination of total phenolics. *Current protocols in food analytical chemistry*. John Wiley & Sons, Inc. New York I1.1.1-I1.1.8.

Yildirim, N. C., Turkoglu, S., Yildirim, N., & Ince, O. K. (2012). Antioxidant properties of wild edible mushroom Pleurotus eryngii collected from tunceli province of turkey. *Digest Journal of Nanomaterials and Biostructures*, *7*, 1647-1654.

Zeković, Z., Vidović, S., & Mujić, I. (2010). Selenium and Zinc content and radical scavenging capacity of edible mushrooms Armilaria mellea and Lycoperdon saccatum. *Croatian Journal of Food Science and Technology*, *2*(2.), 16-21.

Summary/Sažetak

Gljive su dobro izbalansirana hrana koja pruža određene prehrambene i zdravstvene pogodnosti za čovjeka. Gljive proizvode mnoge vrste bioaktivnih spojeva, uglavnom povezanih sa micelama ćelijskog zida, koji pomažu u jačanju sposobnosti imunološkog sistema da se bori protiv kancerogenih tvari. Da bi se razmotrila važnost polifenolnih supstanci i njihovo prisustvo u različitim vrstama gljiva, određena je ukupna antioksidativna aktivnost suhog vrgnja, bijelih i smeđih šampinjona i šitaki gljiva sa bosanskog tržišta. Sadržaj ukupnih fenola je izražen kao ekvivalent galne kiseline /g spektrofotometrijski metodom po Folin-Ciocalteu-u. Sadržaj ukupnih antocijanina je analiziran spektrofotometrijski pH-diferencijalnom metodom na valnim dužinama 525 i 700 nm. Antiradikalna aktivnost (RSA) ekstrakata gljiva je određena DPPH metodom. Analize su pokazale da se sadržaj ukupnih fenola kreće u rasponu od 4.94 mg GAE/g u bukovačama do 35.56 mg GAE/g u uzorku suhog vrgnja. Procenat inhibicije je bio najveći za smeđe šampinjone 88.33 %, a najmanji za bukovače 43.88 %. Gljive ispitane u ovoj studij predstavljaju lako pristupačan izvor antioksidanata.

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2015

44

9-16

Print ISSN: 0367-4444
Online ISSN: 2232-7266

UDC: _____

Original Scientific Article

Investigating the optimum fineness of the coal grain in the Brown Coal Mine Kakanj in order to fully exploit its calorific value

Avdić, N.[a], Šehagić, I.[a]

[a] *University of Sarajevo, Faculty of Science, Department of Chemistry, Zmaja od Bosne 33-35, 71000 Sarajevo, B&H*

[b] *Brown Coal Mine Kakanj, Alije Izetbegovića broj 17, 72240 Kakanj, B&H*

Article info
Received: 11/5/2015
Accepted: 28/5/2015

Keywords:
coal,
tailing, ash,
higher and lower calorific value,
granulometric (grain size) analysis,
chemical analysis

***Corresponding author:**
E-mail: technoprocur@yahoo.com

Abstract: In an array of mechanical operations undertaken during the processing of raw coal at the reloading system of the "Vrtlište" open-cut mining in the brown coal mine Kakanj, the tendency is to separate the combustible from the non-combustible parts of coal all with the aim of obtaining a product with higher calorific value and fewer ash components. Depending on the physical and chemical composition of the raw material and the purpose of the obtained product, our objective was to determine the optimal size of the coal grain in the "Brown Coal Mine Kakanj" whereby the most agreeable aspect in terms of the energy value was to separate the valuable fractions from the tailings. It was established that the top-quality granulation in the raw material being delivered to the client in terms of its energy characteristics is -40+35 mm with a calorific value of 16918 kJ/kg followed by -35+20 and -20 +10 with a calorific value of 13035 kJ/kg and 13819 kJ/kg respectively. They generate the smallest amounts of ash after combustion while the free, hygro and total moisture have the lowest values in these samples.

INTRODUCTION

Safe delivery of adequate selection and quality of coal from the "Vrlište" open-cut mine of the Brown Coal Mine "Kakanj" for the requirements of the "Kakanj" thermal power plant imposed the need to build a coal reception, preparation and wagon loading system at the "Vrlište" open-cut mine whereby the client requested, among other features, that the coal is to be < 40 mm in size.

The granulometric composition of raw coal from the "Vrlište" open-cut mine ranges from 0 mm to a maximum of 400 mm in size. Coal exploitation from the "Vrlište" open-cut mine is conducted on the surface while the exploitation system consists of the removal and depositing of the coal overburden layers and coal exploitation. Due to the geological composition of the coal seams, it is not possible to obtain pure coal and manual separation of tailing is thus conducted within this coal preparation system.

EXPERIMENTAL

Sampling

For the purpose of implementing the experimental part of this paper, sampling of coal was performed on March 23, 2013 at three locations within the coal reception, preparation and wagon loading system. It was established that the coal samples taken from three different locations differed in their granulometric composition and contained tailings. The method used for sampling and processing of the basic coal sample is included in the BAS ISO 5069-1:2002 standard. The calculation of variances for the sampling method was performed in line with the ASTM D 2234-00 method.

An outline drawing of the reloading system including the sampling location is presented in Figure 1. Location (1) is the rubber belt conveyor in the tailing selection room immediately before the manual selection of tailing. Location (2) is also a rubber belt conveyor in the tailing selection room but after the manual selection of tailing and before the entry into a grinder. The coal at the locations (1) and (2) was taken from the +40 mm sieve and the granulation ranges from 40 mm to 400 mm with somewhat smaller fractions created by the movement and inter-crushing of the material.

Location (3) is a rubber belt conveyor that takes on the sieved and the crushed coal from the grinder and transports it to a closed depot. The granulation of the coal sampled at Location (3) ranges from 0 mm to 40 mm.

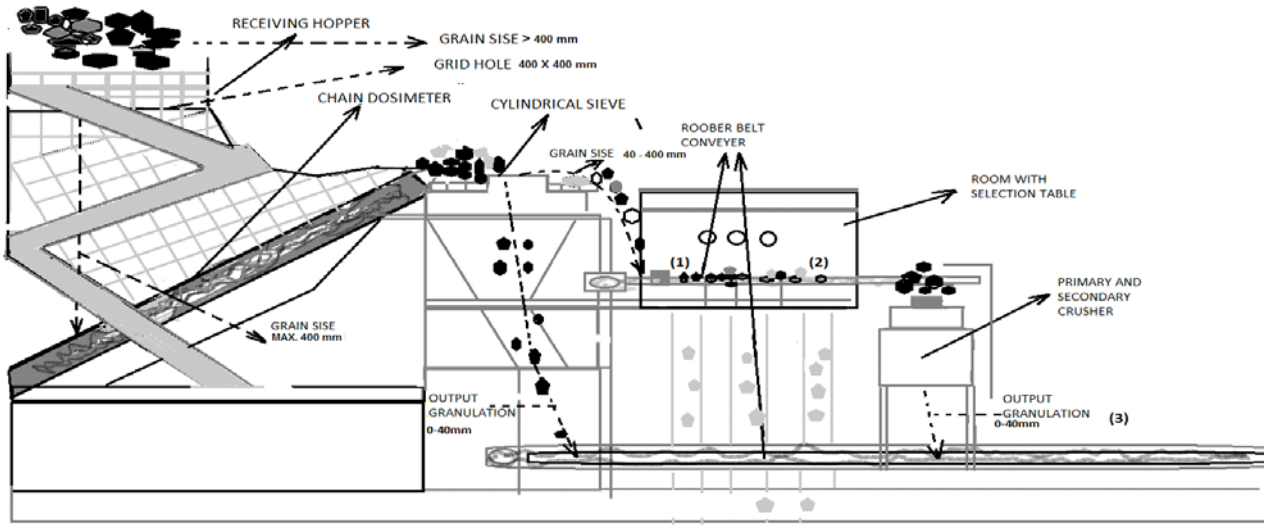


Figure 1: An outline drawing of the reloading system for coal reception, preparation and wagon loading including sampling locations (1), (2) and (3).

Grain-size analysis of samples

The grain-size analysis of coal was conducted on sieves with 400 mm, 250 mm, 100 mm, 50 mm openings and granulation of coal varying between 40 mm to 400 mm.

For 0 mm to 40 mm coal granulation, the following openings of sieves were used: 40 mm, 35 mm, 30 mm, 20 mm, 10 mm, 5 mm, 3 mm and 1 mm.

Physical and chemical analysis of sieves

The methods used for physical and chemical analysis of coal samples were in line with internationally recognized standards: BAS ISO 5069 2:2002; ISO 5068 2:2009; ISO 1171:1999; ISO 1928:2010 and are described in the operational instructions of the "Brown Coal Mine Kakanj."

Tables 1, 2, 3 and 4 present the physical and chemical composition of 40 mm to 400 mm grain-size coal immediately before manual separation of tailing, the same granulation after manual separation of tailing and 0-40 mm granulation after grinding.

The method applied in analysing coal for carbon, hydrogen and nitrogen components by means of a LECO CHN-100 Element Analyser produced by „LECO Corporation" Michigan, USA is part of an internationally recognized standard ASTM D 5373:2008. Identifying the higher calorific value of coal was conducted by a C 5000 Calorimeter manufactured by „IKA-Werke GmbH & Co. KG, Germany while the lower calorific value was established through calculations.

RESULTS AND DISCUSSION

Physical and chemical composition of 40 mm to 400 mm coal fractions prior to manual separation of tailing

Table 1 indicates that the coal sample of 40 mm to 400 mm granulation taken from the sampling location (1), in our case coal containing tailing immediately before its manual separation, is most abundantly present in grains smaller than 250 mm and larger than 100 mm.

Tiny grains of coal smaller than 1 mm and 50 mm that were created by crushing of coal during its transportation as a result of mechanical effects are the least represented.

Table 1 indicates that grains smaller than 250 mm and larger than 100 mm contain the least amount of ash and the highest amount of combustible materials carrying the highest lower calorific value. Coal grains smaller than 100 mm and larger than 50 mm create the largest amount of ash during combustion and have the least amount of combustible material that consequently results in the smallest lower calorific value.

In coal grain samples ranging from 40 mm to 400 mm in size that contain the same amount of tailing after the grinding process until analytical sampling, we can perceive almost the same moderate amount of total moisture in all obtained granulation samples, from which we can conclude that that the differences in calorific values of samples do not originate from the variable content of moisture but rather from the differences in the amount of tailing.

Grains smaller than 50 mm in size have uniform amounts of ash generated by combustion with a tendency for further size degradation which reflects in almost the same lower calorific value as smaller granulations as indicated in Table 1.

Sudden reduction of the lower calorific value for coal grains larger than 50 mm and smaller than 100 mm can be explained by the increased content of tailing in the sample which consequently also means higher amounts of ash after combustion. Grains smaller than 100 mm and larger than 50 mm contain higher amounts of tailing embedded in the pieces of coal which represents a mixture of coal and tailing for these types of granulations.

Volatile materials are most widely present in grains smaller than 250 mm and larger than 100 mm which was expected due to the high amount of combustible materials in these grain samples.

Table 1. *Results of a physical and chemical analysis of 40 mm to 400 mm grain size of coal before manual separation of tailing*

Sieve opening size (mm)	Fraction involvement (%)	Free moisture (%)	Hygroscopic moisture (%)	Total moisture (%)	Ash (%)	Combustible material (%)	Composition of volatile material (%)	Lower calorific value of coal (kJ/kg)
- 400 + 250 mm	6,35	4,80	4,36	9,16	45,42	45,42	25,39	12384
-250 + 100 mm	77,86	4,50	4,24	8,74	39,77	51,49	26,90	14057
- 100 + 50 mm	14,62	5,70	3,00	8,70	57,06	34,24	19,05	8479
- 50 mm	0,50	5,80	3,10	8,90	50,56	40,54	23,32	11659
- 1 mm	0,67	5,86	3,48	9,34	49,89	40,77	23,66	11711
Mean value	63,67	4,71	4,06	8,77	42,78	48,45	25,62	13108

Physical and chemical composition of 40 mm to 400 mm coal fractions after manual separation of tailing

Coal grain samples of 40 to 400 mm in size from the sampling location (2) i.e. coal from which the tailing was separated manually, is most widely present in granulations smaller than 250 mm and larger than 100 mm in size, as indicated by Table 2. The least present granulations are the ones smaller than 1 mm and smaller than 50 mm that were created by mechanical fragmentation of larger pieces of coal as a result of inter-collision of pieces at the sieves and during transportation.

The chemical analysis of various coal grain samples that were partially separated from tailing by means of manual separation indicated that grains smaller than 250 mm and larger than 100 mm have significantly reduced amount of ash (39.77%) in comparison to 51.47% as was established in sample 1, an increased amount of volatile material and the highest lower calorific value. After the separation of tailing coal grains smaller than 100 mm and larger than 50 mm generate significantly smaller amounts of ash during combustion and have larger amounts of volatile material which consequently results in an increased lower calorific value in comparison to the sample with tailing inclusive (Table 1, Table 2).

The total moisture in coal grain samples of 40 to 4000 mm in size indicates to a slight increase in the amount of moisture in smaller-sized grain samples. Its larger presence may be the result of moisture absorption on a now larger area created by fragmentation but also the increased presence of tailing which carries moisture in itself, as evident from an increased amount of ash in grains smaller than 50 mm and 1 mm.

Coal grain samples smaller than 50 mm contain a larger amount of ash generated by coal combustion which is the result of a higher percentage of this fraction in the sample that was created by mechanical shocks on the sieves during longer transportation than with sample 1. It is also the result of a release of small tailing fractions from larger pieces of coal and their sinking through the sieves which brings about a decrease in the calorific value of the sample thus attesting to the lower quality of coal in this fraction in view of its lower calorific value (Table 2). The small amount of ash that was created by combustion is almost uniformly present in granulations smaller than 400 mm and larger 250 mm as well as in grains smaller than 100 mm and larger than 50 mm in size. The smallest amount of ash after combustion was identified in the coal grains smaller than 250 mm and larger than 100 mm.

Table 2. *Results of granulometric and physical and chemical analysis of coal grains of 40 mm to 400 mm in size after manual separation of tailing*

Sieve opening size (mm)	Fraction involvement (%)	Free moisture (%)	Hygroscopic moisture (%)	Total moisture (%)	Ash (%)	Combustible material (%)	Composition of volatile material (%)	Lower calorific value of coal (kJ/kg)
- 400 + 250 mm	31,45	3,20	4,55	7,75	39,32	52,93	28,79	15292
-250 + 100 mm	36,54	2,90	5,28	8,18	22,55	69,27	35,23	21709
- 100 + 50 mm	28,41	3,80	4,86	8,66	38,73	52,61	27,95	15693
- 50 mm	2,12	6,00	2,50	8,50	53,45	38,05	23,03	10601
-1 mm	1,47	6,50	3,13	9,63	49,62	40,75	22,55	11697
Mean value		3,37	4,84	8,21	33,48	58,32	30,69	17599

Considering that this sample contains the optimum amount of moisture and a large amount of combustible material, we can foresee that this coal sample releases the most thermal energy during combustion. The reduction of the lower calorific value in the smaller coal grains can hereby be justified by the increase of tailing reported through the amount of ash in the sample.

The amount of volatile material in coal grains of 40 mm to 400 mm in size following annual separation of tailing is reduced with the reduction in the size of the coal grain. Taking into consideration the results of the physical and chemical analysis of various coal grain samples (Table 2), we can perceive that there is a reduction of the lower calorific value with smaller coal grain samples. We can conclude that the isolated, smaller fractions carry in this phase a larger amount of tailing which as a result of its geological composition binds smaller amounts of organic material and water. In addition to the organic material that encompasses this volatile phase, the carbon mass also includes a significant amount of water that files the carbon mass pores which would not be feasible in the argilic tailing without such a developed porous system.

Physical and chemical composition of coal fractions of 0 to 40 mm in size

The largest number of coal samples that were taken at location (3) i.e. from the conveyor belt after the crushing of coal to a smaller granulation, is comprised of grains smaller than 40 mm and larger than 35 mm. Smaller grains are individually present in small numbers while the least present granulations are those smaller than 3 mm and larger than 1 mm (Table 3).

The physical and chemical analysis of coal grain samples undergoing a reloading system at the open-cut mine "Vrtlište" (Table 3) indicates that the grains smaller than 40 mm and larger than 35 mm contain significantly less ash and respectively more combustible material and have the highest lower calorific value. Coal grains smaller than 3 mm and larger than 1 mm in size generate more ash during combustion thus contain less combustible material and have smaller lower calorific value.

The amount of total moisture is slightly increased in the smaller grain samples. The total moisture together with the ash contributes to the non-combustible part of the coal and heat consumption. Therefore, the smaller coal grain samples in this case have a smaller calorific value.

It is evident that larger amounts of ash generated by coal combustion are present in smaller grain samples, which indicates to a smaller amount of combustible material and thus smaller lower calorific value. (Table 3)

Table 3. *Results of granulometric and physical and chemical analysis of coal grains of 0 mm to 40 mm in size*

Sieve opening size (mm)	Fraction involvement (%)	Free moisture (%)	Hygroscopic moisture (%)	Total moisture (%)	Ash (%)	Combustible material (%)	Composition of volatile material (%)	Lower calorific value of coal (kJ/kg)
- 40 + 35 mm	57,41	4,70	4,53	9,23	32,93	57,84	29,68	16918
-35 + 30 mm	6,95	6,20	4,43	10,63	42,16	47,21	25,36	13035
- 30 + 20 mm	14,84	7,80	3,84	11,64	38,58	49,78	26,19	13819
- 20 + 10 mm	14,01	9,60	3,00	12,60	45,08	42,32	22,53	11243
- 10 + 5 mm	3,15	9,85	1,64	11,49	43,78	44,73	23,25	11909
- 5 + 3 mm	1,60	10,24	1,66	11,90	43,89	44,21	22,57	11876
- 3 + 1 mm	0,33	10,61	1,78	12,39	48,10	39,51	20,67	10149
- 1 mm	1,71	10,33	1,23	11,56	48,65	39,79	20,58	10304
Mean value		6,32	4,00	10,32	36,23	52,73	27,36	15019

Physical and chemical analysis of coal grains for carbon, hydrogen and nitrogen substances

The percentage of carbon is smaller in samples that contain less combustible materials and have a smaller lower calorific value.

The amount of hydrogen as well as nitrogen is also smaller in samples with less combustible material in comparison to the samples with higher presence of combustible material (Table 4).

Table 4. *Results of a chemical analysis of specific coal grains for the percentage of carbon, hydrogen and nitrogen obtained through a CHN-Element Analyser prior to and following the separation of tailing*

Sieve opening size (mm)	Free moisture (%)	Hygroscopic moisture (%)	Total moisture (%)	Ash (%)	Combustible material (%)	Lower calorific value of coal (kJ/kg)	C (%)	H (%)	N (%)
Coal grain sample (-250 + 100) extracted from the basic coal granulation of 40 mm to 400 mm prior to separation of tailing	4,5	4,24	8,74	39,77	51,49	14057	35,6	2,82	0,59
Coal grain sample (-250 + 100) extracted from the basic coal granulation of 40 mm to 400 mm following separation of tailing	2,90	5,28	8,18	22,55	69,27	21709	54,2	3,88	0,84
Coal grain sample (-100+50) extracted from the basic coal granulation of 40 mm to 400 mm prior to separation of tailing	5,70	3,00	8,70	57,06	34,24	8479	21,9	2,00	0,43
Coal grain sample (-100+50) extracted from the basic coal granulation of 40 mm to 400 mm following separation of tailing	3,80	4,86	8,66	38,73	52,61	15693	38,7	3,13	0,65
Coal grain sample (-50+20) extracted from the basic coal granulation of 40 mm to 400 mm prior to separation of tailing	5,80	3,10	8,90	50,60	40,54	11659	29,7	2,37	0,51
Coal grain sample (-50+20) extracted from the basic coal granulation of 40 mm to 400 mm following separation of tailing	6,00	2,50	8,50	53,45	38,05	10601	26,5	2,26	0,47
Coal grain sample (-50+20) extracted from the basic coal granulation of 0 mm to 40 mm	9,60	3,00	12,60	45,80	42,34	11243	29,3	2,26	0,52

For grains smaller than 250 mm and larger than 100 mm in size and for grains smaller than 100 mm and larger than 50 mm in size, the amount of carbon is smaller in samples where the tailing has been separated. There is also a slight increase in the amount of carbon for grains smaller than 50 mm in size that have been freed of tailing in comparison to the samples containing tailing.

The amount of hydrogen for grain samples less than 250 mm and more than 10 mm and for granulations smaller than 100 mm and larger than 50 mm is insignificantly smaller for samples that have not been subjected to tailing extraction.

Almost the same amount of hydrogen is evident in granulations smaller than 50 mm for samples that have been separated from tailing and samples of the same grain size that still contain tailing.

For grains smaller than 250 mm and larger than 100 mm in size and for grains smaller than 100 mm and larger than 50 mm in size, the amount of nitrogen is smaller in samples where tailing has not been extracted. Moreover, smaller amount of nitrogen is present in grains smaller than 50 mm in size for the sample that has been separated of tailing in comparison to the samples of the same grain size that still contain tailing.

CONCLUSION

The optimum granulation regarding energy characteristics is -40+35 mm with a calorific value of 16918 kJ/kg followed by -35+20 and -20+10 with a calorific value of 13035 kJ/kg and 13819 kJ/kg respectively. They generate the least amount of ash after combustion while the free, hygro and total moisture is also the lowest in these samples.

The moisture present in other samples is approximately the same and its amount is subjected to change in the granulations depending on air humidity and porosity or grain composition. From this we can conclude that its effect on the calorific value of coal will be constant for the listed granulations and that the changes in the coal grain energy value are caused by variable amount of tailing.
Grains smaller than 250 mm and larger than 100 mm in size have the largest amount of volatile and combustible material which results in the largest separation of organic material i.e. hydrogen and organic nitrogen.
Considering the fact that as Table 2 indicated, the largest amount of ash after manual separation of tailing is to be found in the grains smaller than 50 mm which are mixed with other coal after grinding to get the same grain size, it is recommended that this grain size is subjected to separation of tailing through a sink-float separation method.
Grains smaller than 250 mm and larger than 100 mm in size are most widely present in the raw coal sample and carry the most combustible material. Therefore, these should be put aside during the separation process and then proceed with fragmentation.

In order to identify the most favourable coal granulations regarding their energy values and environmental effects during combustion, it is important to carry out an analysis of the content and form of sulphur in individual granulations and investigate the possibility of extracting these fractions during the process of separation.
It is undoubtedly necessary to analyse the economic validity of specific granulation treatments before they are brought down to grains smaller than 40 mm in size or before the whole grain smaller than 40 mm in size is subjected to some form of the applicable separation methods in order to maximise the release of tailing. In addition to an increase of energy value of the raw material, this would reduce transport costs to the supplier as well as the costs of ash treatment after combustion and boost the conditions for better environmental protection.

REFERENCES

American society for Testingh and Materials ASTM (2000). ASTMD 2234-00, Standard Pratctice for Collection of sample of Coal. West Conshocken, United States: 100Barr Harbor drive

American society for Testingh and Materials ASTM (2008). ASTMD7373-2008, StandardStandard Test Medodhes for Instrumental Determination of Carbon, Hydrogen,and nitrogen in Laboratory Samples of Coal and Coke. West Conshocken, United States: 100 Barr Harbor drive

Institut za standardizaciju Bosne i Hercegovine BAS (2002). BAS ISO 5069-1:2002 (bs), Smeđi ugljevi i ligniti: Principi uzorkovanja-Dio 1: Uzorkovanje za određivanje sadržaja vlage za opća ispitivanja = Brown coals and lignites – Principels of samling – Part1: Sample preparation for determination of moisture content and for general analysis. Istočno Sarajevo: BAS

Institut za standardizaciju Bosne i Hercegovine BAS (2002). BAS ISO 5069-1:2002 (bs), Smeđi ugljevi i ligniti: Principi uzorkovanja-Dio 2: Priprema uzorka za određivanje sadržaja vlage za opća ispitivanja = Brown coals and lignites – Principels of samling – Part2: Sample preparation for determination of moisture content and for general analysis. Istočno Sarajevo: BAS

International Standards Office (2009). ISO 5068 – 2: 2009 (E), Brown coals and lignites-determination of moisture content – Part 2: Indirect gravimetric method for moisture in the analysis sample. Geneve: ISO

International Standards Office (1999). ISO 1171: 1999 (E), Solid minera fuels – Determination of ash content, Geneve: ISO

International Standards Office (2010). ISO 1982: 2010 (E), Solid minera fuels – Determination of gross calorific value by bomb calorimetric method and calculation of net calorific value, Geneve: ISO

Sredojević, J. i sar. 2010. Dopunski rudarski projekat postrojenja za prijem pripremu i utovar uglja u vagone na pogonu PK „Vrtlište“, RMU „Kakanj“, Kakanj

Sulejmanović S. (2006). Idejni projekat postrojenja za prijem, pripremu i pretovar uglja u vagone na PK „Vrtlište“: Rudarsko – tehnološki dio. Kakanj:RMU „Kakanj“

Summary/Sažetak

U nizu mehaničkih operacija kroz koje prolazi oplemenjivanje sirovog uglja, na pretovarnom sistemu površinskog kopa „Vrtlište“ rudnika Kakanj teži se razdvojiti gorivi od negorivog dijela uglja, a sve u cilju dobivanja proizvoda koji će imati veću toplotnu vrijednost, a manji sadržaj komponenata pepela. U zavisnosti od fizičko-hemijskog sastava sirovine i namjene dobivenog proizvoda, cilj nam je bio odrediti optimalnu veličinu zrna uglja " Rudnika Kakanj" pri kojoj je razdvajanje korisnih od jalovinskih komponenata najpovoljnije u pogledu energetske vrijednosti.

Utvrđeno je da je u sirovini koja se isporučuje kupcu najkvalitetnija granulacija u pogledu energetskih karakteristika jeste -40+35 mm sa toplotnom vrijednoću od 16918 kJ/kg a slijede je -35+20 i -20+10 sa toplotnim vrijednostima 13035 kJ/kg i 13819 kJ/kg, respektivno. Iz njih nastaje i najmanja količina pepela nakon izgaranja, dok su gruba, higro i ukupna vlaga takođe najniže u ovim uzorcima.

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2015

44

Print ISSN: 0367-4444
Online ISSN: 2232-7266

UDC: _____

Original Scientific Article

17-22

The effects of problem-based learning on students' achievements in primary school chemistry

Zejnilagić-Hajrić, M.*, Šabeta, A., Nuić, I.

University of Sarajevo, Faculty of Science, Department of Chemistry, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina

Article info
Received: 20/3/2015
Accepted: 16/6/2015

Keywords:
problem-based learning,
students' achievements,
pre-post-test design,
primary school chemistry

***Corresponding author:**
E-mail: mzejnilagic@pmf.unsa.ba
Phone: 00-387-33-279864
Fax: 00-387-33-649359

Abstract: Specific applications of cognitive and constructivist theories in problem-based learning (PBL) include connecting prior knowledge and skills with new information. This prominent instructional method is widely accepted in higher education around the world, but it also shows good results when applied in primary education of various disciplines. This paper presents effects of PBL application in 8th grade primary school chemistry when learning about chemical compounds, using questionnaires and tests of knowledge in pretest-posttest study with control (CG) and experimental (EG) groups. Students in CG were taught in usual way with teacher-centered approach, while in EG the PBL materials designed for the purpose of this study were applied. Results showed (1) significant improvement of students' achievements in EG, (2) these students are not used to this teaching method so they encountered certain difficulties, (3) overall interest and engagement in chemistry lessons has increased.

INTRODUCTION

Many authors agree that teaching methods which allow active participation of students in the teaching process result in better achievements and overall learning results. One of those methods is problem-based learning (PBL).
Problem-based learning (PBL) is an instructional method aimed at preparing students for real-world settings. By requiring students to solve problems, PBL enhances students' learning outcomes by promoting their abilities and skills in applying knowledge, solving problems, practicing higher order thinking, and self-directing their own learning (Jonassen and Hung, 2012). PBL was implemented in medical school programmes in the 1950s for the first time, in response to students' unsatisfactory performances due to the emphasis on memorization of fragmented biomedical knowledge (Barrows and Tamblyn, 1980). Since then, it has been modified and applied in various professional areas (Yoon, Woo, Treagust, *et al*., 2014), among them in science (Duch, Groh and Allen, 2001) and education (Peterson and Treagust, 1998).

PBL implies learning during problem solving – students focus on a simple or complex problem which does not have only one correct answer readily available from textbook (Hmelo-Silver, 2004). Students can learn individually or divided in groups. Accent is set on "what" to learn to successfully solve the problem (Artino, 2008).
Application of PBL in science courses can be more efficient if it includes some components of scientific processes and science concepts (Gallagher *et al*., 1995). Gallagher *et al.* (1995) suggested four essential elements for PBL in science:

(a) problems should focus on significant science concepts,
(b) there should be opportunities to test students' ideas through experiment or fieldwork,
(c) students should manage their own data,
(d) and the presentation of their solutions.

PBL is conceptually based upon the cognitive and constructivist theories. Their specific applications in PBL include connecting new information with prior knowledge, elaboration and construction of information learned and collaborative learning. Students' learning is

initiated by a need to solve an authentic problem. In PBL, students are no longer receiving the learning content from the instructor in a "textbook" logical sequence (Jonassen and Hung, 2012).

Problem solving promotes learners' higher-level thinking skills, and consequently, results in deeper understanding and better application of the knowledge in the future. It is challenging and motivating. This intrinsic motivational component helps increase students' desire to learn and sustains their interest throughout the course of the learning.

Traditional instruction usually presents content information with context-free problems. The main shortage of traditional methods is the lack of connection between knowledge learned and real-life practice. As stated in US National Science Education Standards (1996, p. 173), "for students to develop the abilities that characterize science as inquiry, they must actively participate in scientific investigations, and they must actually use the cognitive and manipulative skills associated with the formulation of scientific explanations". Students tend to develop algorithmic rather than cognitive skills, which leaves student with no choice but memorizing algorithms if they want to survive chemistry course (Cracolice, Deming and Ehlert, 2008).

Many empirical studies were testing the effectiveness of PBL in various contexts and the general conclusion is that PBL enhances students' problem solving, higher order thinking, self-directed learning skills, and motivation to learn. Also, PBL students consistently outperformed traditional students on long-term retention assessments (Jonassen and Hung, 2012).

PBL can be effectively applied in chemistry education, especially in laboratory part of courses. The laboratory is an important component of science education that can foster positive attitudes and interest towards science. Students can learn not only scientific concepts, but also scientific thinking abilities, and experimental skills (Yoon, Woo, Treagust, *et al*., 2014). PBL is an alternative to typical laboratory instructional methods because as it can resolve its several shortcomings (Arnold, 2003; Hicks and Bevsek, 2012; Kelly and Finlayson, 2007; Ram, 1999).

RESEARCH METHODOLOGY

This pretest-posttest study was conducted during April and May 2012. It included control (CG) and experimental (EG) groups, questionnaires and tests of knowledge as instruments for collecting data. Participants were 8th grade primary school students (n=51) from one school in Sarajevo, divided in two groups equal by their knowledge of chemistry at the beginning of this study.

Study included four major teaching units in primary school chemistry: Oxides, Acids, Bases, and Salts.

Students in CG were taught in usual way with teacher-centered approach with demonstration when applicable, while in EG the PBL materials designed for the purpose of this study were applied. EG students were working in groups and teacher served as facilitator of the learning process.

For topic Oxides students were asked to propose and to perform an experiment to prove that people exhale carbon dioxide using glass, plastic straw and water solution of calcium hydroxide. After defining oxides as group of chemical compounds, teacher demonstrated burning of magnesium strip and students were asked to divide oxides into groups.

Acids – based on PowerPoint presentation on acid rain, students needed to conclude how the rain becomes acidic, which chemical processes happen in the atmosphere? After group experiment on the properties of indicator in acidic solutions, students defined acids and their properties, acid dissociation and its examples.

Knowledge test T1 is used in order to establish prior knowledge, and final test of knowledge identified students' achievements. Both test were the same for both groups and made using textbooks for primary school and were comprised out of tasks and questions corresponding to the age of participants.

Questionnaires were used to identify students' most common difficulties in learning chemistry, to get insight into frequency of PBL application in teaching chemistry and (for EG), opinion on PBL method applied in their classes.

Research hypotheses

Based on research of relevant literature on problem-based learning, the main hypothesis set was:

H: PBL is more effective in teaching chemistry at primary school than usual teaching methods based on teacher-centered approach.

Sub-hypotheses were also set in order to verify and support main hypotheses:

SH1: Students perceive learning chemistry as more difficult and demanding than other teaching subjects

SH2: Students are more interested in PBL than usual teaching methods

SH3: Students have certain difficulties in finding solution to the problem

SH4: Students have considerable experience in PBL during their education – considering that their primary education lasts for nine years.

SH5: PBL supports students' engagement in teaching chemistry

Instruments for collecting data

Test of knowledge T1 was used to explore students' knowledge on fundamental chemical concepts relevant for learning and understanding selected teaching section involving four topics. Moreover, it was needed to establish whether these two groups were equal by their knowledge on the beginning of the study.

Test of knowledge T2 was used to explore students' knowledge on the topics that were taught using PBL in EG and using usual teacher centered approach in CG.

Questionnaires for students of experimental (QS_{EG}) and control (QS_{CG}) group containing questions about students' perceptions on chemistry as science, teaching and learning chemistry and problem-based learning.

RESULTS AND DISCUSSION

Students' achievements on entry knowledge test (T1) with no statistically significant difference (M_{CG}=2.72, SD_{CG}=0.64; M_{EG}=2.80, SD_{EG}=0.63) indicated that our participants have comparable knowledge on fundamental chemical concepts important for this study. This fact is also obvious in Figure 1. showing marks of these students they earned on this test of knowledge.

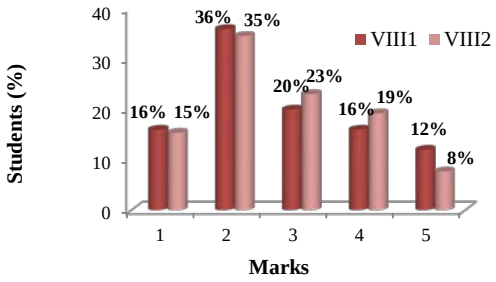


Figure 1. Students' achievements on T1

After teaching all four topics, results of a knowledge test (T2) showed statistically significant difference in students' achievements in EG compared to CG (M_{CG}=2,84, SD_{CG}=0,64; M_{EG}=3,84, SD_{EG}=0,72). Students' marks are shown on Figure 2.

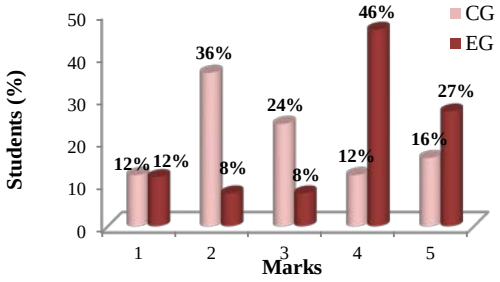


Figure 2. Students' achievements on T2

As shown on Figure 2, greatest differences are noted when considering mark 2: 36% of CG students and only 8% of EG students were awarded with mark 2. When considering marks 4 and 5, only 28% of CG student earned these marks, but 73% of EG student for marks 4 and 5 show promising effects of PBL application when teaching these concepts in primary school. It should be noted that results of a questionnaire showed that this was the first time that these students encountered with PBL, so better results could be expected with continuous application of PBL.

In order to illustrate the headway of EG student, we have shown the comparison of EG students' results on T1 and T2.

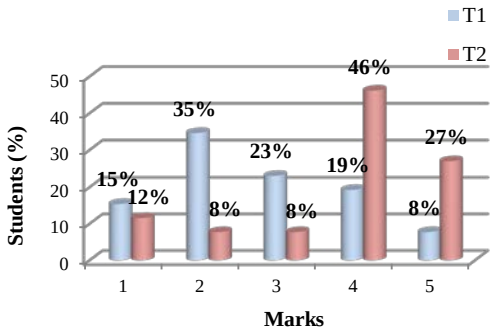


Figure 3. Comparison of EG students' achievements on T1 and T2

There is obvious difference between students' achievements before and after teaching using PBL method. We have tested different concepts with these two tests – we have compared them because marks are much higher when PBL was applied, disregarding the teaching content.

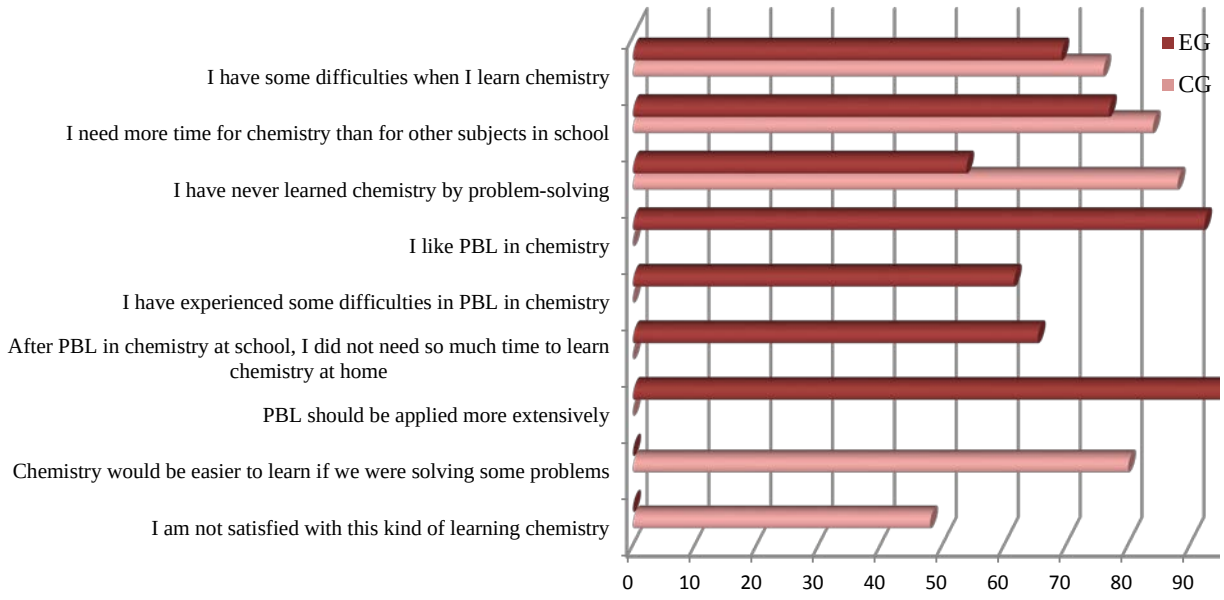


Figure 4: Selected data from questionnaire

Overall results of a questionnaire showed that students are interested in PBL method, but they do encounter certain difficulties when finding a solution to problem.
Items in questionnaire were designed in order to test sub-hypotheses.
SH1 was confirmed because a majority of students perceive chemistry as a difficult subject (CG: 76%, EG 69.2%), that they need more time to learn for than some other subjects in school (CG:84%, EG: 76,9%).
SH2 was also confirmed since majority of EG students liked PBL application during teaching sequence (100%), and consider that PBL should be applied more extensively (100%). CG students were not familiar with PBL but they were not satisfied with the current way of teaching and learning chemistry (88%).
EG student were also asked if they are familiar with PBL in other subjects. Even though curricula for primary school went through modernization few years ago, from 8-year to 9-year long primary school, PBL is not instructional method that is extensively applied during primary school. Therefore, 61.5% of EG students experienced some problems in finding solution to problem in PBL, which confirmed SH3.
As stated above, it was expected for "new" curriculum to be changed not only in themes and subjects, but also in new teaching methods that were not represented before. This is in accordance with Bodner (1992), who suggested that changing the curriculum may not be enough, proposing that the methods of curriculum delivery must also be changed. These teaching methods are not new, but they were not used sufficiently. This sub-hypothesis (SH4) therefore was not confirmed since 88% of CG students said that they never solve problems during learning chemistry, and 92% that they never (or rarely) do that when learning other subjects; EG students' responses were similar: 92.3% do not (or rarely do) learn chemistry by solving problems, and 88.5% on other subjects in school.
Last sub-hypothesis was also confirmed since the teacher noticed overall greater engagement and involvement of her student in learning chemistry, but also their statements from questionnaire: 52% of CG student are not involved into the teaching process, 80% of EG students believe that chemistry would be easier to learn if problem solving was represented, while 65.4% needed less time for learning chemistry at home after PBL application.

CONCLUSION

Results of this study, comparable to findings of foreign authors, showed the benefits of problem-based learning as teaching method in primary school chemistry. The main hypothesis, that a problem-based learning is more efficient than conventional teaching methods in chemistry (based on teacher-centered approach) is confirmed. Also, we have encountered some difficulties in using this method, primarily due to its rare application in primary school teaching generally. We believe that if teachers applied this method more extensively, these problems would be minimized, while both students and their teachers would benefit from it.

REFERENCES

Arnold, R.J. (2003). The Water Project: A Multi-Week Laboratory Project for Undergraduate Analytical Chemistry. *Journal of Chemical Education* 80 (1), 58-60

Artino, R.A. (2008). *A Brief Analysis of Research on Problem-Based Learning*. University of Connecticut.

Barrows, H.S., Tamblyn, R.M. (1980). *Problem-Based Learning – An Approach to Medical Education*. Springer

Bodner, G.M. (1992). Why Changing The Curriculum May Not Be Enough. *Journal of Chemical Education* 69 (3), 186-190

Cracolice, S. M., Deming, C. J., Ehlert, B. (2008). Concept Learning versus Problem Solving: A Cognitive Difference. *Journal of Chemical Education* 85 (6), 873-878.

Duch, B.J., Groh, S.E., Allen, D.E. (2001). *The Power of Problem-Based Learning: A Practical „how to" for Teaching Undergraduate Courses in Any Discipline*. Stylus Publishing, LLC.

Gallagher, S. A., Stepien, W. J., Sher, B. T., Workman, D. (1995). Implementing Problem-Based Learning in Science Classrooms. *School Science and Mathematics*, 95 (3), 136-146.

Hicks, R.W., Bevsek, H.M. (2012) Utilizing Problem-Based Learning in Qualitative Analysis Lab Experiments. *Journal of Chemical Education* 89 (2), 254-257.

Hmelo-Silver, C.E. (2004). Problem-Based Learning: What and How Do Students Learn? *Educational Psychology Review* 16 (3), 235-266.

Jonassen D.H., Hung, W. (2012). Problem-Based Learning. In: Seel, N. M. (Ed.), *Encyclopedia of the Sciences of Learning*, (p.p. 2687-2690). Springer.

Kelly, O.C., Finlayson, O.E. (2007). Providing solutions through problem-based learning for the undergraduate 1st year chemistry laboratory. *Chemistry Education Research and Practice* 8 (3), 347-361.

National Research Council (1996). *National Science Education Standards.* National Academies Press.

Peterson, R.F., Treagust, D.F. (1998). Learning to Teach Primary Science through Problem-Based Learning. *Science Education* 82 (2), 215-237

Ram, P. (1999). Problem-Based Learning in Undergraduate Education: A Sophomore Chemistry Laboratory. *Journal of Chemical Education* 76 (8), 1122-1126

Yoon, H., Woo, A. J., Treagust, D.F., Chandrasegaran, A.L. (2014). The Efficacy of Problem-based Learning in an Analytical Laboratory Course for Pre-service Chemistry Teachers. *International Journal of Science Education*, 36 (1), 79-102.

Summary/Sažetak

Specifične primjene kognitivne i konstruktivističke teorije u problemskom učenju (engl. Problem-based learning, PBL) uključuju povezivanje prethodno stečenog znanja i vještina s novim informacijama. Ova istaknuta nastavna metoda je široko prihvaćena u visokom obrazovanju širom svijeta, a također pokazuje dobre rezultate i kada se primjenjuje u osnovnoj školi u različitim nastavnim predmetima. U ovom radu prikazani su efekti primjene problemske nastave u nastavi hemije u osnovnoj školi prilikom poučavanja hemijskih spojeva u osmom razredu osnovne škole, upotrebnom anketnih upitnika i testova znanja u predtest-posttest istraživanju koje je uključivalo kontrolnu (CG) i eksperimentalnu (EG) grupu. Učenici kontrolne grupe poučavani su uobičajenim načinom, frontalnim oblikom rada, dok su učenici eksperimentalne grupe bili poučavani problemskim pristupom, primjenom nastavnih materijala dizajniranih za potrebe ovog istraživanja. Rezultati su pokazali (1) značajno poboljšanje postignuća učenika u EG, (2) učenici nisu navikli na primjenu ove nastavne metode te su se susretali s određenim poteškoćama, (3) opći interes i zalaganje u nastavi hemije su porasli.

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2015

44

23-28

Print ISSN: 0367-4444
Online ISSN: 2232-7266

UDC: _____

Original Scientific Article

Dependance of viscoelastic properties of two emulsion formulations on preparation process

Elezović, A.[a*], Kostić, S.[b], Hadžiabdić, J.[a], Rahić, O.[a], Vranić, E.[a], Vehabović, M.[b]

[a]Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Sarajevo, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina

[b]Bosnalijek, d.d. Sarajevo, Jukićeva 53, 71000 Sarajevo, Bosnia and Herzegovina

Article info
Received: 31/10/2014
Accepted: 25/5/2015

Keywords:
rheology, viscoelastic properties, cosmetic emulsions

***Corresponding author:**
E-mail: alisakaric@yahoo.com
Phone: 00-387-33-586173
Fax: 00-387-33-586183

Abstract: Rheological properties are crucial for cosmetic formulations, determining product's properties during production and application. O/W emulsions (pH 6.86–7.10) were prepared with decyl-oleate as internal phase. A-formulations contained K-stearate, while N-formulations contained polyglyceryl-stearate and -behenate as principal emulsifiers. The formulations were prepared by adding the water to the oil phase (NA and AA) or vice versa (NB and AB). Oscillatory measurements were performed on Haake RheoStress using double gap cylinder. In amplitude sweep at low stresses all samples behaved as viscoelastic solids. With increasing stress, phase angles increased to >80°. Crossover of storage and loss moduli for AA happened at nine times higher stress compared to the other formulations, forming lamellar crystalline gel network. Linear viscoelastic region showed that AA was much more stable. Frequency sweep showed NA and AB to be liquid-like. In NB and AA decrease in complex viscosity indicates better spreadability. Stability of phase angles and storage and loss moduli indicate more elastic behavior. New nonionic emulsifier was more independent of processing, unlike anionic emulsifier. However, AA formulation gives much better feel properties, needed in cosmetic formulations.

INTRODUCTION

One of the major groups of topical formulations are emulsions, consisting of two immiscible liquid phases (water and oil) in which one phase is dispersed in the other and stabilized by emulsifier. Emulsions are thermodynamically metastable systems. They are exposed to physical, chemical and microbiological influences during manufacture, transport, storage and use that can induce changes in the emulsion. Very different emulsion structures can be achieved depending on the emulsifiers used and their concentration. Also, the production process affects the end product, since different droplet sizes and droplet size distributions can be achieved. This all affects not only microstructure and stability of an emulsion, but also macrostructure and how the product appeals to the consumer.

Studies of rheological properties are crucial for liquid and semisolid pharmaceutical and cosmetic formulations, because they determine product's properties during production and application. However, rheological studies for regulatory purposes are used only to a certain degree, remaining at the level of viscosity determination (Podczeck, 2007). Studies of viscoelastic characteristics have great potential in development and optimization of stability as well as sensory properties of topical formulations (Adeyeye, Jain, Ghorab *et al*., 2002; Ibanescu, Danu, Nanu *et al*., 2010; Moravkova and Filip, 2013).

Emulsions show, like all real materials, viscoelastic flow characteristics, displaying both viscous (liquid-like) and elastic (solid-like) behavior (Đaković, 2006; Podczeck, 2007). These characteristics are studied using oscillatory measurements (rotor of the rheometer goes back and forth at an angular frequency and amplitude or oscillates).

In an ideal elastic material, deformation is proportional to the load, and the force (shear stress, τ) is greatest at maximum deformation. The stress and strain curves will be in phase (phase angle (δ) equals 0°) for a sinusoidal load (Fig. 1(a)) (Brummer, 2006).

In an ideal viscous material, there is proportionality of shear stress and shear rate, *i.e.* maximum shear rates correspond to maximum forces. There is a phase lag of 90° between shear rate and the strain (Fig. 1(c)) (Brummer, 2006).

Between these extremes lie viscoelastic materials. Here, the phase angle or lag of shear stress (τ) and the strain (γ) of the material is between the extreme values ($0^o > \delta > 90^o$) (Fig. 1(b)), depending on the nature of the material, *i.e.* is it more elastic or viscous.

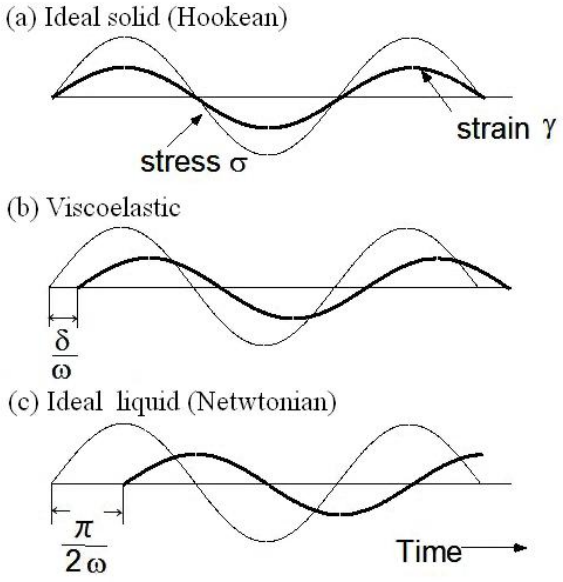


Figure 1: Relationship between applied stress and strain of the material and phase angle for (a) ideally elastic (solid) material, (b) viscoelastic and (c) ideally viscous (liquid) material.

The parameters of viscoelastic materials are usually frequency dependent. Elastic component of a material is described by storage modulus (G'), while the viscous component is described by loss modulus (G''). They are related to phase angle as follows:

$$\tan\delta = \frac{G''}{G'} \qquad (1)$$

$$G^* = \frac{\tau}{\gamma} = G' + iG'' \qquad (2)$$

G* is complex modulus which is a measure of material's overall resistance to deformation and $i = \sqrt{-1}$.

Complex dynamic viscosity can be calculated:

$$\eta^* = \frac{G^*}{i\omega} = \frac{G''}{\omega} - \frac{G'}{\omega} \qquad (3)$$

ω is angular frequency (Brummer, 2006).

Viscoelastic properties are studied using dynamic oscillatory measurements, which are done without destroying material's structure. These measurements include oscillatory amplitude sweep, in which frequency is kept constant and the amplitude of deformation changes, *i.e.* the strain increases. In oscillatory frequency sweep the amplitude is kept constant and the frequency changes.

The aim was to study viscoelastic properties of O/W emulsions prepared in two different manners using either nonionic or anionic emulsifier system.

EXPERIMENTAL

Preparation of emulsion formulations

Decyl oleate, cetostearyl alcohol, glyceryl monostearate and stearic acid were obtained from Caesar & Loretz GmbH, Germany. Propylparaben and methylparaben were obtained from Sigma-Aldrich Inc., USA. TEGO® Care PBS 6 (polyglyceryl stearate and behenate) was kindly provided by FC Franken-KosmetikChemie-Handel GmbH & Co.KC, Germany and Evonik Industries AG, Germany. These chemicals were used as received. Potassium hydroxyde was from Kemika Zagreb, Croatia, and was of p.a. quality. Deionized water was produced on Milli-Q Water Purification System (Millipore Corporation, USA).

Four formulations of O/W emulsions were prepared with composition shown in Table I. Formulations NA and NB contained nonionic principal emulsifier (TEGO® Care PBS 6), while formulations AA and AB contained anionic principal emulsifier (potassium stearate).

Table I: Composition of the formulations

Excipient	*NA and NB*	*AA and AB*
A		
Decyl oleate	25.5%	25.5%
Stearic acid	-	3.0%
TEGO® Care PBS 6	3.0%	-
Cetostearyl alcohol	0.5%	0.5%
Gliceryl monostearate	0.5%	0.5%
Propylparaben	0.05%	0.05%
B		
Disodium-EDTA	0.1%	0.1%
Potassium hydroxyde	-	0.12%
Methylparaben	0.3%	0.3%
Deionized water q.s. ad	100.0%	100.0%

The formulations were prepared by heating the oil phase (A) and the water phase (B) to 80ºC separately (until clear), and than mixed in two manners:

1. by adding the water phase (B) to the oil phase (A), resulting in phase inversion as the formulation is stirred (200 rpm) and cooled to room temperature (NA and AA formulations)

2. by adding the oil phase (A) to the water phase (B) and stirring (200 rpm) until the mixture is cooled to room temperature, which is the standard way of preparing stearate creams (NB and AB formulations).

pH measurements

The pH of the formulations was measured directly using Orion Star A211 (ThermoScientific, USA).

Oscillatory measurements

Oscillatory measurements were performed on Haake RheoStress (ThermoScientific, USA) using double gap sensory geometry with 5.100 mm gap.
Amplitude sweep was carried out during 100 s, with τ ranging from 0.01 through 1000.0 Pa at frequency of 1.000 Hz at 23ºC.

Frequency sweep was carried out at τ = 1.0 Pa with changing frequency from 10.0 to 0.01 Hz at 23ºC.
All results were processed using software HAAKE RheoWin (ThermoScientific, USA).

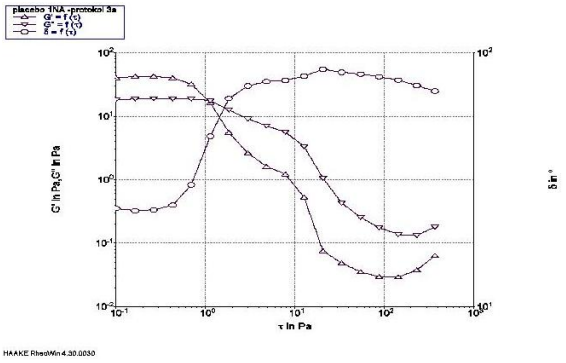
RESULTS AND DISCUSION

The results of pH measurements of emulsion formulations are shown in Table II.

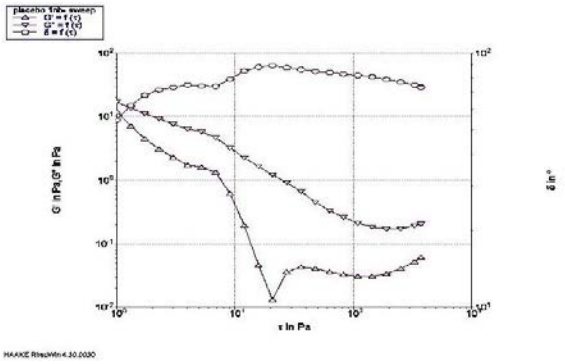
Table II: Measured pH values of the formulations

Formulation	pH value
NA	6.91
NB	6.86
AA	6.90
AB	7.10

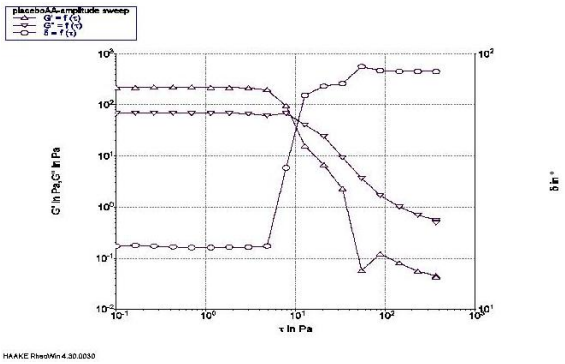
The results were all very near pH 7, thus the adjustment of pH was deemed unnecessary.



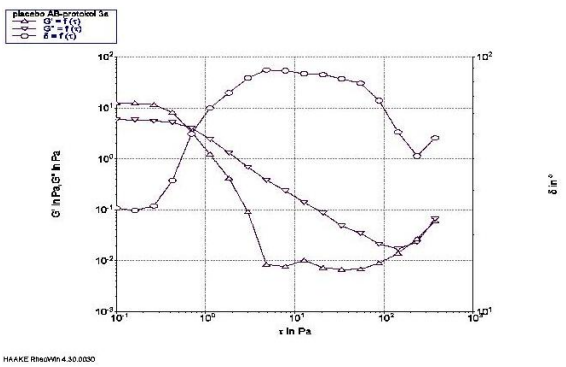
(a)



(b)



(c)



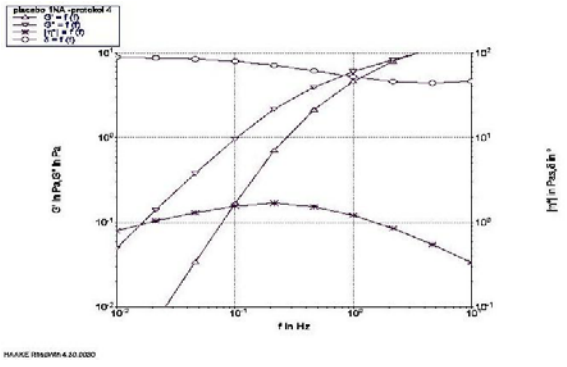
(d)

Figure 2: Amplitude sweep for formulations NA (a), NB (b), AA (c), AB (d). The storage modulus (△ – G'), loss modulus (▽ – G'') and phase angle (○ – δ) are functions of shear stress (τ).

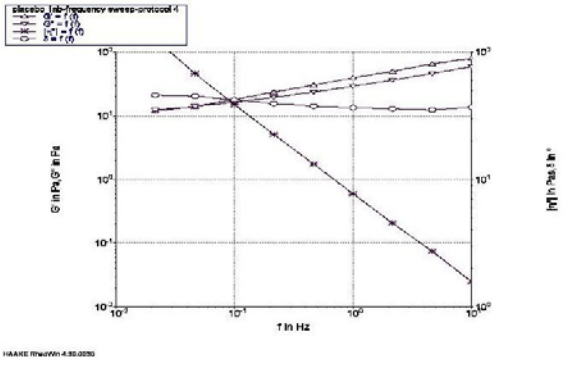
Oscillatory amplitude sweep showed (Fig. 2) that all samples behaved as elastic solids at low shear stress (0.1 Pa), since G' > G''. With increasing stress, the internal structure breaks down. The region of stable structure (linear viscoelastic region or LVER) differed. LVER for sample NA ended at τ = 0.7 Pa, and for NB sample at τ = 0.43 Pa, indicating somewhat more stable structure in NA sample, compared to NB. In sample AB the structure broke down at 0.26 Pa, while in AA the structure this happened at 4.83 Pa. This shows very large dependence on production processing in this type of formulation.

As the internal structure broke down at higher stress values, the crossover of storage (G') and loss (G'') moduli occurred, and formulations became more viscous than elastic, *i.e.* more liquid-like. This happened for all but one sample on low shear stresses (τ_{NA} = 1.080 Pa, τ_{NB} = 1.349 Pa, τ_{AB} = 0.620 Pa). Sample AA had crossover at τ_{AA} = 9.220 Pa. Crossover at lower stress levels indicates easier spreadability of lotions on skin (Ibanescu, Danu, Nanu *et al.*, 2010), which might be the case with formulations NA, NB and AB. On the other hand, sample AA displayed more stable and resilient structure, thus harder spreadability, but richer feel (Ibanescu, Danu, Nanu *et al.*, 2010).

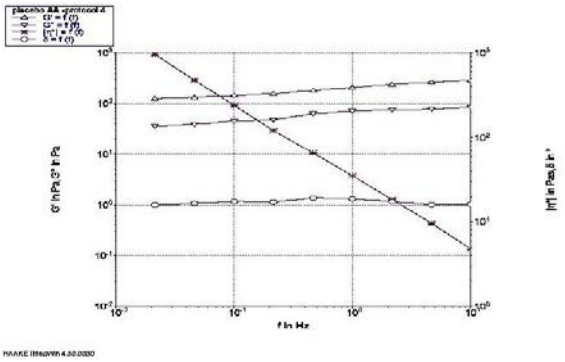
In all samples, phase angle (δ) rose with increasing shear stress, but at low end of τ was the smallest for AA sample. As structures of formulations broke down (beyond LVER) phase angles increased to >85°, also consistent with samples becoming liquid-like.
LVER, G', G'' and δ values indicate much more stable structure in formulation AA compared to the classic emulsions and formation of lamellar crystalline gel network phase in this sample.



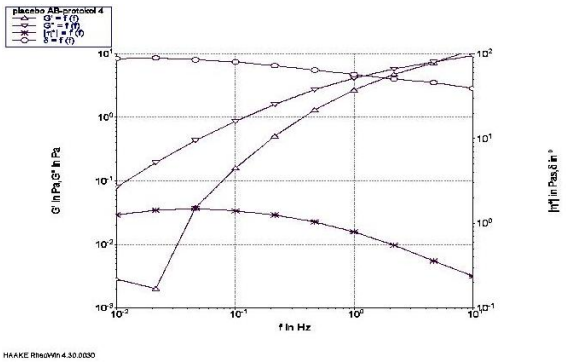
(a)



(b)



(c)



(d)

Figure 3: Frequency sweep for formulations NA (a), NB (b), AA (c), AB (d). The storage modulus (△ – G'), loss modulus (▽ – G''), phase angle (○ – δ) and complex viscosity (∗ –η*) are functions of frequency (f).

Oscillatory frequency sweep showed (Fig. 3) that sample NA behaved as a viscoelastic liquid, since $\delta \sim 80^o$, G'' > G', with crossover at high frequency (6.514 Hz), and complex viscosity (η*) decreased at the frequency region of the crossover of moduli. Sample NB, on the other hand, showed more elastic behavior compared to NA, since $\delta \sim 40^o$, at low frequency G'' > G', but crossover happened at 0.04554 Hz, and at higher frequencies G' > G''. As the frequency increased complex viscosity fell logarithmically.
AA sample behaved as a viscoelastic solid. Its phase angle was constant at $\delta \sim 17^o$, G' > G''. The values of storage and loss moduli, without crossover in the studied frequency region, were higher than for sample NB, as well as complex viscosity, indicating more stabile gel structure. Complex viscosity logarithmically fell. Since at rest complex viscosity was high, and phase angle was stable and low, it implies rich feel or texture of the cream (Ibanescu, Danu, Nanu *et al.*, 2010).
AB sample behaved as a liquid, since its phase angle was $\delta \sim 80 - 90^o$, G'' > G', with crossover at frequency of 5.052 Hz. The complex viscosity (η*) decreased at the frequencies of the crossover of moduli.

CONCLUSION

The differences in viscoelastic properties of nonionic (N) formulations were not as pronounced as in anionic (A) formulations with regard to the preparation process. Nonionic emulsifier used in N formulations is new one, designed for low viscosity cosmetic preparations. It is much more independent of processing compared to the much older anionic stearate emulsifier. On the other hand, AA sample obtained with phase inversion gave much better feel properties, very needed in cosmetic formulations.

ACKNOWLEDGEMENT

The authors wish to thank FC Franken-Kosmetik Chemie-Handel GmbH & Co.KC, Germany and Evonik Industries AG, Germany for kindly providing TEGO® Care PBS 6. Many thanks to Mr. Neil Cunningham for taking time to revise the manuscript.

REFERENCES

Adeyeye, M.C., Jain, A.C., Ghorab, M.K.M., Reilly, W.J.Jr. (2002). Viscoelastic evaluation of topical creams containing microcrystalline cellulose/sodium carboxymethyl cellulose as stabilizer. *AAPS PharmSciTech*, 3(2), article 8, 1-10.

Brummer, R. (2006). *Rheology Essentials of Cosmetic and Food Emulsions*. Springer.

Đaković, Lj. (2006). *Koloidna hemija*. Zavod za udžbenike – Beograd.

Ibanescu, C., Danu, M., Nanu, A., Lungu, M., Simionescu, B.C. (2010). Stability of disperse systems estimated using rheological oscillatory shear tests. *Revue Roumaine de Chimie*, 55 (11-12), 933-940.

Moravkova, T., Filip, P. (2013). The influence of emulsifier on rheological and sensory properties of cosmetic lotions. Advances in Materials Science and Engineering, 2013, 1-7.

Podczeck, F. (2007). Rheology of Pharmaceutical Systems. In Swarbrick, J. (Ed.), *Encyclopedia of Pharmaceutical Technology*. (3rd Ed.) (p.p. 31280-03146). Informa Healthcare USA, Inc.

Summary/Sažetak

Reološke osobine su ključne za kozmetičke formulacije, budući da određuju osobine proizvoda tokom njegove proizvodnje i aplikacije. U/V emulzije (pH 6.86–7.10) su pripremljene sa decil-oleatom kao unutrašnjom fazom. A-formulacije su sadržavale K-stearat, a N-formulacije poligliceril-stearat i –behenat, kao glavne emulgatore. Formulacije su pripremljene dodajući vodenu u masnu fazu (NA i AA) ili obrnuto (NB i AB). Oscilatorna mjerenja su izvršena na Haake RheoStress korištenjem cilindra sa duplim zazorom. Pri promjeni amplitude, kod niskih napona smicanja svi uzorci su se ponašali kao viskoelastična čvrsta tijela. Povećanjem napona smicanja, fazni uglovi su se povećali do >80°. Križanje modula elastičnosti i viskoznosti za AA se desilo pri devet puta većem naponu smicanja u poređenju sa ostalim formulacijama, te formira lamelarnu kristalnu gel mrežu. Linearni viskoelastični region je pokazao AA kao mnogo stabilniju. Promjena frekvencije je pokazala da su NA i AB slične tečnostima. Kod NB i AA smanjenje kompleksnog viskoziteta indicira bolju razmazivost. Stabilnost faznog ugla i moduli elastičnosti i viskoznosti ukazuju na više elastično ponašanje. Novi neionski emulgator je bio više neovisan o metodi izrade za razliku od anionskog emulgatora. Međutim, AA formulacija daje mnogo bolje osjetne osobine potrebne u kozmetičkim formulacijama.

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2015

44

29-32

Print ISSN: 0367-4444
Online ISSN: 2232-7266

UDC: _____

Original Scientific Article

Spectrophotometric determination of total iron content in black tea

Mandal, Š.[a] *, Banjanin, B.[b], Kujović, I.[a], Malenica, M.[c]

[a]*Department of Natural Sciencies in Pharmacy, Faculty of Pharmacy, University of Sarajevo, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina*

[b]*Department of Organic chemistry, Faculty of Health Travnik, University of Travnik, Slavka Gavrančića 17c, 72270 Travnik, Bosnia and Herzegovina*

[c]*Department of Biochemistry and Clinical Analysis, Faculty of Pharmacy, University of Sarajevo, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina*

Article info
Received: 31/10/2014
Accepted: 25/5/2015

Keywords:
iron,
black tea,
spectrophotometry,
mineral digestion

***Corresponding author:**
ŠaćiraMandal
E-mail: shakira.mandal@gmail.com
Phone: 00-387-33-586189
Cell: 00-387-61-614203

Abstract: The aim of this work was the assessment of total iron (Fe) content in some black tea brands using mineral digestion and spectrophotometric method. Four samples of black tea from different manufactures in three parallels were prepared by digestion and oxidation with a mixture of sulphuric and nitric acid. The total Fe content in analyzed black tea varies from 21.3 mgFe/kg to 37.6 mg Fe/kg. The used spectrophotometric method is simple and sensitive method that can be applied for the determination of total Fe content in plant material.

INTRODUCTION

Tea is one of the most popular beverages consumed worldwide. Tea infusion is made from the processed leaves of the plants *Camellia sinensis L., familia Thecea* and the three most popular types of tea (green, oolong, and black) are distinguished on the basis of degree of fermentation. The leaves of green tea are dried and roasted but not fermented, whereas black tea leaves are additionally fermented. The chemical composition of tea leaves consists of tanninic substances, flavonols, alkaloids, proteins and amino-acids, enzymes, aroma-forming substances, vitamins, minerals, and trace elements (Jha et al**.,** 1996).

Among the minerals and essential trace elements that are essential to human health, Ca, Na, K, Mg, and Mn are present in tea leaves at g/kg level, while Cr, Fe, Co, Ni, Cu, Zn are present at mg/kg level (Cao et al., 1998; Fernandez-Caceresetal., 2001). Plants obtain these trace elements from growth media such as nutrient solutions and soils. The extent to which they take up metals depends on the extent to which trace elements are bound to soil constituents and the other sources include pesticides and fertilizers.

Habitual drinking of tea infusions may significantly contribute to daily dietary requirements for specific elements. The total contents of metals in tea leaves differ according to the type of tea (green or black) and are probably influenced by many other factors, e.g. soil properties. Iron, one of the most abundant metals on Earth, is essential to most life forms, and to normal human physiology (Dobrinas et al., 2011; Jha et al., 1996; Mose et al., 2014). Iron is an integral part of many proteins and enzymes that maintain good health. In human metabolism, iron is an essential component of proteins involved in oxygen transport. It is also essential for the regulation of cell growth and differentiation. A deficiency of iron limits oxygen delivery to cells, resulting in fatigue, poor work performance, and decreased immunity. On the other hand, excess amounts of iron can result in toxicity and even death (Moroydor Derun et al., 2012). Many studies have concluded that tea has numerous beneficial effects on health, including the prevention of many diseases such as skin cancer,

diabetes, Parkinson's disease, myocardial infarction, and coronary artery disease (Schwalfenberg et al. 2013). The knowledge of both micronutrients and toxic elements content in beverages is important, taking into account nutrition requirement and intoxication risk related with their consumption (Afsana et al., 2004; Powell et al. 1998; Nelson M. and Poulter J., 2004; Temme E.H.M. and Van Hoydonck P.G.A., 2002).
The aim of this work was the assessment of total iron (Fe) content in some black tea brands using mineral digestion and spectrophotometric method.

EXPERIMENTAL

Biological material

Plant samples (black tea leaves) - Ceylon Tea (Turkey), Hazir Harman Çay (Turkey), Indian black tea (Franck Zagreb, Croatia) and Ceylon Tea (Emona Brand extra quality, Kosovë).
Four plant samples were prepared by wet digestion (open system) where weighed mass of 1.0000 g was heated with mixture of concentrated nitric acid and sulphuric acid for 4 hours at temperatures 110-130^{0}C. Solid phase separated by filtration (blue filtered paper), dissolved in mixture of nitric and hydrochloric acids (concentration of 0.05mol/L, 1:1 v/v), transferred in volumetric flask (100mL) and diluted to mark with same mixture of acids. Digestion of each samples was done in triplicate.

Spectrophotometric method

Spectrophotometric method was performed with a Genesys 2 UV- VIS Spectrometer, Model TM2. Solutions of prepared twenty samples were yellow to pale red color. Before spectrophotometric analysis, intensity of color was increased by addition of potassium thiocyanate (Sigma-Aldrich Co. LLC) for complexation of iron ions and formation of red complex with different composition from $[FeSCN(H_2O)_5]^{2+}$ to $[Fe(SCN)_6]^{3-}$ (Itodo et al., 2012; Paul E. Adams, 1995). Standard stock solutions of iron(III) ions was prepared by dissolving 20 mg of iron (III) chloride (Sigma-Aldrich Co. LLC) in 100 ml deionized water in a volumetric flask (100 ml). The calibration solutions were prepared by pipetting volumes of 0.05, 0.10, 0.25, 0.50, 0.75, 1.00, 1.50, 2.00 and 2.50 ml, respectively of the stock standard solution into volumetric flasks (10 ml). Next, of volumes of 1.00 ml of nitric acid and 1.20 ml of potassium thiocyanate (concentration of both solution was 5 M) were added to each volumetric flasks to obtain a concentration range from 1.40 to 69.81 µg/ml Fe. The absorbance of each solution (working and analyzed solutions) was measured at absorption maximum of 481.0 nm using 10 mm quartz cuvette (Figure 1).

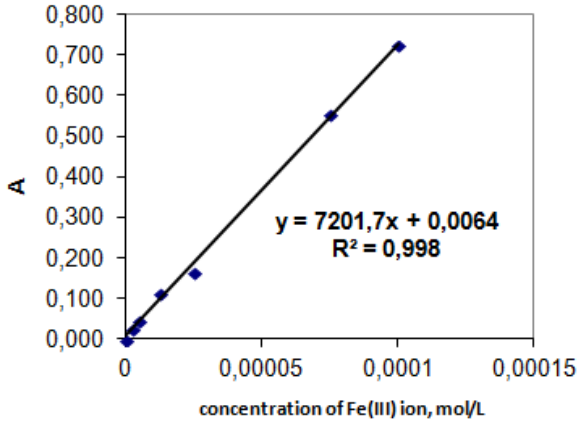


Figure 1. Standard calibration curve

RESULTS AND DISCUSSION

The total Fe content after mineral digestion determined by using an spectrophotometric method in different brands of black tea is shown in Table 1.

Table 1. Total content of iron in analyzed black teas (**mean±S.E.M**)

Sample	Black tea	Total Fe content (mg/kg)
I	Ceylon Tea	37.6±1,13
II	Hazir Harman Çay	21.3±0,88
III	Indian black tea	29.01±0,37
IV	Ceylon Tea-extra quality	27.4±0,98

The result shows that these four brands of black tea product contained Fe concentration ranging from 21.3 to 37.6 mg/kg with a mean value of 28.8 mg/kg. The lowest concentration (21.3 mg/kg) was observed in Hazir Harman Çay tea brand and the highest concentration (37.6mg/kg) was observed in Ceylon Tea brand.
A number and different instrumental techniques, such as induced coupled plasma-optical emission spectroscopy (ICP-OES), induced coupled plasma-mass spectrometry (ICP-MS), flame atomic absorption spectrophotometry (AAS), etc. (Moroydor Derun et al., 2012; Marbaniang et al., 2011;Achudume and Owoeye, 2010; Moseti et al., 2013; Gebretsadik and Chandravanshi, 2010),are used for determination of minerals and essential trace elements in plant and soil. The reason for such a number of methods is that no single method fulfills all the conditions, such as precision, accuracy, selectivity, speed, etc. In all methods the sample must be degraded by wet or dry digestion. In order to quantify the use of any of the above mentioned methods, iron must be converted into the appropriate form (to be oxidized to iron (III) or iron (II)). Conversion of iron in ferri and ferro ions and decomposition of the sample is carried out with a mixture of concentrated acids H_2SO_4/HNO_3. Problem with digestion is that, when sample is heated in such acidic conditions, Fe may evaporate in the form of its volatile compounds, so it is

very important to control heat to avoid evaporation of Fe (Moroydor Derun et al., 2012;Achudume and Owoeye, 2010; Street et al., 2006). Also, some quantity of iron precipitated and is lost during degradation and oxidation of biological material. Determination of level of iron in plant material is influenced by many factors.. The preparation method (time duration of digestion, temperature, etc.) has also a great influence (Marcus 1996). Tea polyphenols have a high affinity for metals and also for biological macromolecules. Many factors may be contributing to the metals accumulation in the tea leaves, such as soil composition, its organic matter contents, manufacturing process and environmental pollutions. It was confirmed that the content of metals might be an adequate discriminator of tea varieties and their geographical origin. The main source of trace elements in plants including tea is their growth media (pH soils), use of fertilizers (e.g. nitrogenous), insecticides and herbicides. Industrialization have also been shown to influence the element content in plants (Fernandez-Caceres et al., 2001; Dobrinas et al., 2011).

A previous report showed that the Fe concentrations in imported teas in Czech Republic varied from 0.037 to 0.142 mg/mL (Street et al., 2006), which are consistent with our results (0.021 mg/mL to 0.376 mg/mL). Our results indicated that Fe contents in our samples were lower than some black teas from the markets of India (Marbaniang et al., 2011) and Nigeria (Achudume and Owoeye, 2010), which were reported to have Fe contents from 0.439 mg/mL and 0.99 to 2.39 mg/mL, respectively. Also, MoroydorDerun and others (2012) found the highest Fe of 2.396±0.040 mg/L in black teas from the market from Turkey.

Many elements present in food at major, minor and trace level are reported to be essential to human's health. Human body requires both metallic and nonmetallic elements for healthy growth, development and the proper functioning of the body. The determination of these elements in beverages and plant is of the most importance and is currently the subject of studies by various researchers (Dobrinas et al., 2011; Mose et al., 2014). Analysis of content of the metals in tea is very important especially determination of their concentrations that need to be presented in properly recommended values. Tea has a recognized therapeutic value. It is important in the prevention and treatment of many diseases (Fernandez-Caceres et al. 2001). The study of trace elements in tea has been taken up as trace elements play an important role in the complex metabolic pathways in human system and their deficiency or excess may cause disease. Identification of metal containing components of tea is not simple due to the analytical difficulties associated with both the separation of such components and their quantitative measurement.

CONCLUSION

These data demonstrate the tea plant's ability to accumulate the studied element (iron) as nutrient, further underlining tea consumption as a potential dietary source of the nutritionally essential inorganic nutrients necessary for various biological processes. The used spectrophotometric method is simple and sensitive method that can be applied for the determination of total Fe content in plant material.

REFERENCES

Achudume C.A., Owoeye D. (2010). Quantitative assessment of heavy metals in some tea marketed in Nigeria-Bioaccumulation of heavy metals in tea. *Health*, 2(9), 1097 –1100.

Adams P.E. (1995).Determining iron content in foods by spectrophotometry.*Journal of Chemical Education*, 72(7), 649 – 651.

Afsana K., Shiga K., Ishizuka S., Hara H. (2004).Reducing effect of ingesting tannic acid on the absorption of iron, but not of zinc, copper and manganese by rats. *Bioscience, Biotechnology and Biochemistry*, 68, 584–592.

Cao X., Zhao G., Yin M., Li J. (1998). Determination of ultratrace rare earth elements by inductively coupled plasma mass spectrometry with microwave digestion and AG50W-x8 cation exchange chromatography. *Analyst*, 123, 1115–1119.

Dobrinas, S., Soceanu, A.D., Stanciu, G., Bratu, S. (2011). Essential elements levels in herbs and their infusions. *Ovidius University Annals of Chemistry*, 22(1), 37-40.

Fernandez-Caceres P.L., Martin M.J., Pablos F., Gonzalez A.G. (2001). Differentiation of tea (Camelliasinensis) varieties and their geographical origin according to their metal content. *JournalofAgricultural and Food Chemistry*, 49, 4775–4779.

Gebretsadik W.D., Chandravanshi S.B. (2010).Levels of metals in commercially available Ethiopian black teas and their infusions.*Bull. Chem. Soc. Ethiop*., 24(3), 339–349.

Itodo A. U., Usman A., Sulaiman S. B., Itodo H. U. (2012). Color Matching Estimation ofIronConcentrationsin Branded Iron Supplements Marketed in Nigeria.*Advances in Analytical Chemistry*, 2(1), 16 – 23.

Jha A., Mann R.S., Balachandran R. (1996). Tea: A refreshing beverage. *Indian Food Industry*, 15, 22–29.

Marbaniang, D. G., Baruah, P., Decruse, R., Dkhar, E. R., Diengdoh, D. F., Nongpiur, C. L. (2011). Study of trace metal (Cr, Mn, Fe, Co, Ni, Cu, Zn and Cd) composition in tea available at Shillong, Meghalaya, India.*International Journal of Environmental Protection*, 1(1), 13-21.

Marcus A., Fisher A., Ree G., Hill J. (1996). Preliminary study using trace element concentrations and a chemometrics approach to determine the geological origin of tea. *Journal of Analytical Atomic Spectrometry*, 113, 521-525.

Mohammed, M.I., Sulaiman, M.A. (2009). Analysis of some metals in some brands of tea sold in Kano, Nigeria. *Bayero Journal of Pure and Applied Sciences*, 2(2), 34-39.

Moroydor Derun, E., Kipcak, A. S., Dere Ozdemir, O., Piskin, M. B. (2012). Cr, Fe and Se Contents of the Turkish Black and Green Teas and the Effect of Lemon Addition.*World Academy of Science, Engineering and Technology*, 6, 11-23.

Mose M.T., Moseti K.O., Wanyoko J.K., Kinyua J.K., Kariuki D., Magiri E.N., Obanda M.A. (2014). Selected Inorganic Nutrients in Black Tea from Three Tea Growing Agro-Ecological Areas in Kenya. *American Journal of Plant Sciences*, 5, 473– 479 .

Moseti, K.O., Kinyanjui, T., Wanyoko, J.K., Kurgat, J.K., Too, J.C., Omondi, K.G., Wachira, F.N. (2013).Fe, Zn, Cu, Pb and Cd in Tea Grown and Marketed in Kenya; A Quantitative Assessment.*International Journal of Environmental Protection*, 3(6), 24–30.

Nelson M., Poulter J. (2004). Impact of tea drinking on iron status in the UK: a review. *Journal of Human Nutrition and Dietetics*, 17, 43–54.

Powell J.J., Trevor J., Burden T.J., Thompson R.P.H. (1998). In vitro mineral availability from digested tea: a rich dietary source of manganese. *Analyst*, 133, 1721–1724.

Schwalfenberg G., Genuis J.S., Rodushkin I. (2013). The Benefits and Risks of Consuming Brewed Tea: Beware of Toxic Element Contamination. *Hindawi Publishing Corporation Journal of Toxicology*, Volume 2013, Article ID 370460, 8 pages.

Street R., Szsáková J., Drábek O., Mládková L. (2006). The status of micronutrients (Cu, Fe, Mn, Zn) in tea and tea infusions in selected samples imported to the Czech Republic. *Czech J. Food Sci*., 24, 62-71.

Temme E.H.M., Van Hoydonck P.G.A. (2002). Tea consumption and iron status. *European Journal of Clinical Nutrition*, 56, 379–386.

Summary/Sažetak

Cilj ovog rada je procjena ukupnog sadržaja željeza (Fe) u nekim brendiranim crnim čajevima primjenom mineralne digestije i spektrofotometrijske metode. 4 uzorka crnog čaja različitih proizvođača i u 3 paralelke pripremljeni su digestijom i oksidacijom sa smjesom sumporne i nitratne kisleine. Sadržaj ukupnog željeza u uzorcima crnog čaja varirao je od 21 mg Fe/kg do 37,6mg Fe/kg. Primjenjena metoda spektrofotometrije je jednostavna i osjetljiva i može biti primjenjena za određivanje ukupnog sadržaja željeza u biljnom materijalu.

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2015

44

Print ISSN: 0367-4444
Online ISSN: 2232-7266

UDC: _____

Original Scientific Article

33-38

Influence of chloride ions on various corrosion resistance of zinc coating

Ličina, S.*, Ostojić, J., Gutić, S., Cacan, M.

University of Sarajevo, Faculty of Science, Department of Chemistry, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina

Article info
Received: 16/4/2015
Accepted: 16/6/2015

Keywords:
Corrosion
Zinc coating
Chloride ions

***Corresponding author:**
Sara Ličina
E-mail: saralicina@hotmail.com
Phone: 061/828 - 333

Abstract: Corrosion is one of the major causes for global crisis concerning the loss of materials resources and energy. It is also the cause of significant economical loss in many countries. Certain measures can be taken to minimize this problem, which is the first and foremost goal of this research work. The objectives of this study are to investigate respective aspects of chloride ions influence on various galvanic properties of zinc coating. Those included: corrosion resistance properties, the effects different concentrations of chloride solutions thereon, also the properties of galvanically deposited zinc in a variety of coating thicknesses with respect to corrosion resistance of galvanically deposited zinc in chloride solutions and the influence of chloride ions on the zinc coating given by different technologies, as warm and galvanic processes, analysis of their properties on corrosion resistance. All tests were performed with potentiostat/galvanostat Model 263A, guided by potentiodynamic polarization and linear polarization method.

Based on the results presented in this research work, it is concluded that despite the costs the corrosion protection is the most effective way to prevent material loss.

INTRODUCTION

Based on the data of industrially developed countries, corrosion damage can get up 1-3% of national income. In some countries, the corrosion damage is included in the values of national currencies. However, this data is quite unreliable since collateral damage (e.g. accidents, loss of life and loss of production) is not correspondingly measured by money (national currency), and often it cannot be even adequately expressed. Complete protection of metals and alloys from corrosion is almost impossible, but certain measures can be taken to minimize the problem, namely: the choice of material, construction methods, change of the environment, coating. (Dobovišek, 1968; Potter, 1968. Franz *et al*., 1980. Jelic *et al*., 2007.)

MATERIALS AND METHODS

The necessary durability of metal structures against corrosion can be determined by studying and defining the basic laws of corrosion processes and also analyising tests of corrosion resistance in a variety of environments. The application of different metals and alloys under unfavorable conditions, which change during exploitation, require testing under different non-destructive and destructive methods in order to evaluate their corrosion resistance. These include: visual inspection, identification of the mechanism and vision and corrosion, determination of the type and composition of the isolated products of corrosion, corrosion rate measurement and penetration depth of damage, loss or gain of mass, examining changes in mechanical properties.

Before each test, it is important to determine the real objectives of the examination, and according to that to make a choice of the most suitable methods for the assessment of damage caused by corrosion.

The purpose of such tests is to solve certain practical problems. (Dobovišek, 1968.; E. Potter C., 1968.; Filipovic, I. *et* Sabioncello, P. 1960; Martinez, S., 2007; Susic, M., V., 1980.)

Performance testing

To test the corrosion resistance of zinc we used five standard solutions of NaCl. Determination of chloride was carried by Mohr procedure and obtained values are presented in Table 1.

Table 1. Concentrations of Cl^- - ions in solutions

MARK SOLUTION	CONCENTRATION of Cl^- ions [g / L]
I	0,02
II	0,03
III	0,05
IV	0,60
V	3,03

All the electrochemical measurements were performed by potentiodynamic and linear polarization method. We used potentiostat/galvanostat Model 263A, connected to the software package Model 270/250 Research Electrochemistry Software 4.30. When performing the tests the temperature was adjusted to a 20°C using RC5 thermostat Lauda. Before each test, the experimental conditions were adjusted accordingly.

All tests were carried out in a standard electrochemical cell, equipped with three electrodes, zinc (electrochemical and chemical process the coating, as well as varying thickness galvanically applied zinc) as the working electrode, silver-silverchloride as the reference electrode and platinum as an auxiliary electrode. Before testing, each electrode had to be adequately prepared.

Auxiliary (platinum) electrode:

Isolated with teflon tape, only the specific surface area had undergone the examination. Electrodes were prepared by sanding the surface with sandpaper of a different fineness (200-600), then polishing it with aluminum oxide powder and finally rinsing it with distilled water.

Working (zinc) electrode:

Isolated with teflon tape, only the specific surface area had undergone the examination, then the surface of electrodes was measured.

The first test was conducted using potentiodynamic polarization method. Measurement has to begin by establishing a stable value of the potential open circuit, i.e.by potential that is 250 mV more negative than the corrosion potential, and ends at a potential that is 250 mV more positive than the corrosion potential.

The testing was then conducted using linear polarization with the same sample. Measurement begins with establishing a stable value of the potential open circuit, the potential that is 30 mV more negative than the corrosion potential, and ends at a potential which is 30 mV more positive than the corrosion potential.

Experimentally obtained values of the current and the potential are shown as semilog E – log *i*, also known as Tafel display.

The obtained values in Tafel diagrams and polarization curves, have helped to determine the corrosion current density, corrosion potential and polarization resistance. Therby the insight on the intensity of the corrosion process is provided as well as the corrosion behavior of a particular material in a particular environment.

After the tests, the preparation is carried out again, and the process is repeated for all samples.

RESULTS AND DISCUSSION

Influence of chloride ions on galvanic zinc coatings

The behavior of galvanic zinc coatings (thickness of about 40 μm (60 min. zinc galvanized)) at different concentrations of chloride-ions in water: 0.02 g / L, 0.03 g / L, 0.05 g / L; 0.60 g / L, 3.03 g / L was analysed

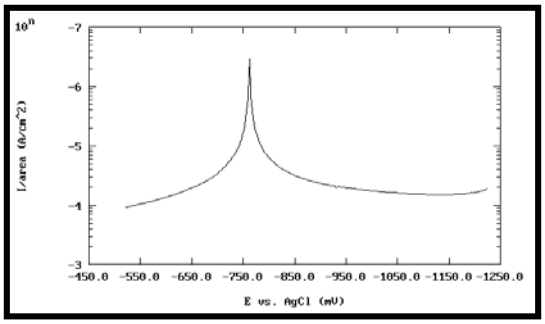


Figure 1. Tafel diagram for galvanic zinc in solution Cl^- - ion concentration. 0.02 g / L

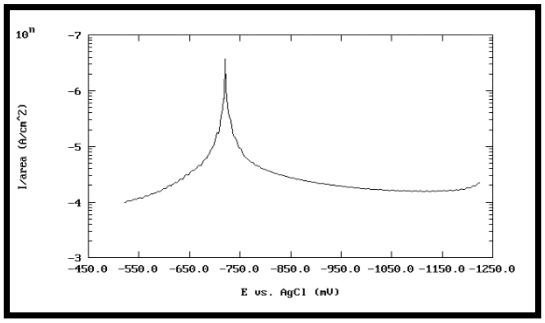


Figure 2. Tafel diagram for galvanic zinc in solution Cl^- - ion concentration. 0.03 g / L

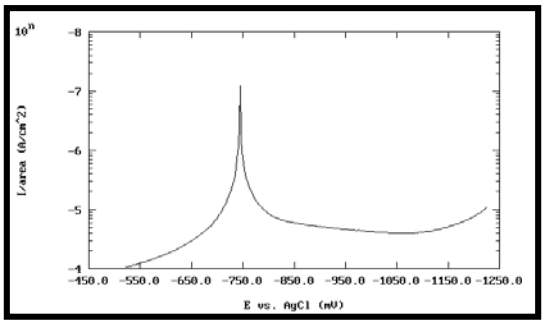


Figure 3. Tafel diagram for galvanic zinc in solution Cl^- - ion concentration. 0.05 g / L

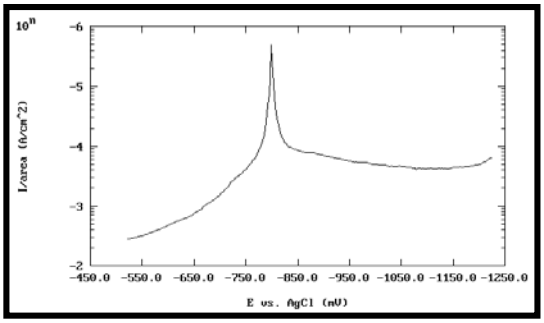


Figure 4. Tafel diagram for galvanic zinc in solution Cl^- - ion concentration. 0.60 g / L

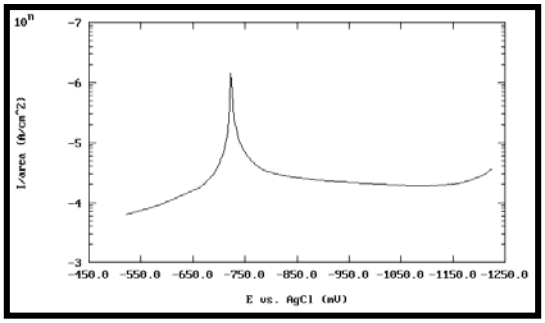


Figure 5. Tafel diagram for galvanic zinc in solution Cl^- - ion concentration. 3.03 g / L

Previously obtained results are presented table 2.

Table 2. Influence of Cl^- - ions on the speed corrosion

CONCENTRATION Cl^- - ions [g / L]	**POTENTIAL CORROSION [mV]**	**POLARIZATION RESISTANCE [mV/A]**	**DENSITY CORROSION CURRENT [mA/cm^2]**	**SPEED CORROSION [mm/yr.]**
0,02	- 740	9 x 10^7	3,66 x 10^{-4}	0,0055
0,03	- 698	8 x 10^7	4,48 x 10^{-4}	0,0067
0,05	- 722	5 x 10^7	8,85 x 10^{-4}	0,0132
0,60	- 776	8 x 10^6	2,15 x 10^{-3}	0,0321
3,03	- 700	2 x 10^6	2,09 x 10^{-2}	0,3126

Influence of thickness of galvanic zinc on the corrosion resistance

The behavior of galvanic zinc coatings of different thickness such as 40 μm (60 min. zinc galvanized), about 30 μm (45 min zinc galvanized), and about 25 μm (30 min. zinc galvanized), in solution Cl^- - ion concentration 0.60 g/L was analysed and presented in Figures 6,7, and 8.

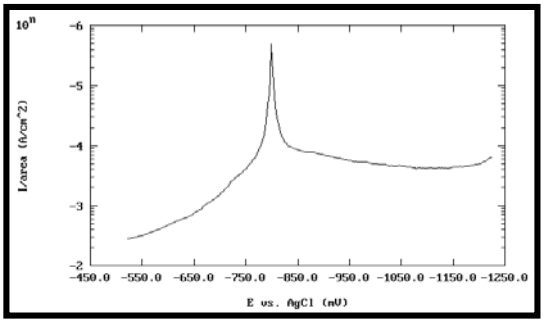


Figure 6. Tafel diagram for galvanic zinc thickness of about 40 μm

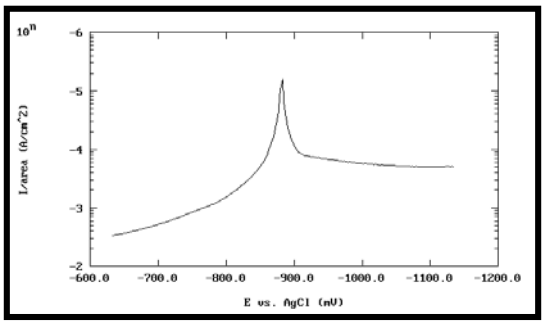


Figure 7. Tafel diagram for galvanic zinc thickness of about 30 μm

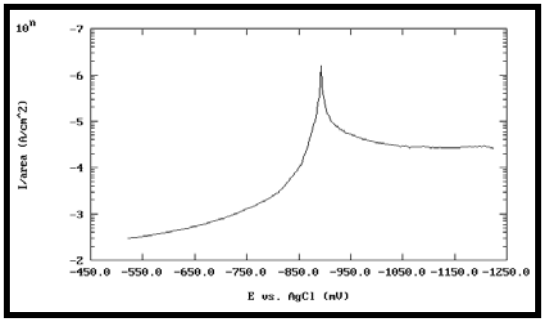


Figure 8. Tafel diagram for galvanic zinc thickness of about 25 μm

Previously obtained results are presented table 3.

Table 3. Influence of coating thickness on the speed corrosion

THICKNESS OF PROTECTION [μm]	POTENTIAL CORROSION [mV]	POLARIZATION RESISTANCE [mV/A]	DENSITY CORROSION CURRENT[mA/cm^2]	SPEED CORROSION [mm/yr.]
about 40	- 776	8 x 10^6	2,15 x 10^{-3}	0,0321
about 30	- 860	7 x 10^6	3,64 x 10^{-3}	0,0545
about 25	- 870	2 x 10^6	1,07 x 10^{-2}	0,1600

Influence of various technologies used for applying zinc coating on the corrosion resistance

The behavior of various technologies used for applying zinc coating (chemical process the coating, thickness coating of about 100μm and electrochemical process the coating, thickness coating of about 40μm) in a solution of Cl^- - ion concentration 0.03 g / L was tested and presented here.

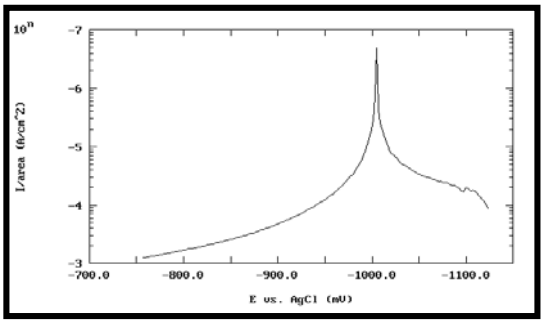


Figure 9. Tafel diagram for chemical process the coating, thickness coating of about 100 μm

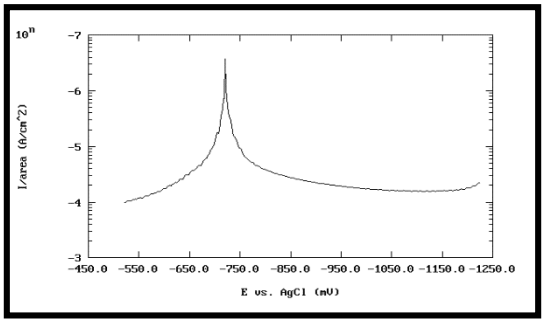


Figure 10. Tafel diagram for electrochemical process the coating, thickness coating of about 40 μm

Previously obtained results are presented in table 4.

Table 4. Influence of technologies coatings on the speed corrosion

APPLICATION OF PROTECTION	POTENTIAL CORROSION [mV]	POLARIZATION RESISTANCE [mV/A]	DENSITY CORROSION CURRENT[mA/cm^2]	SPEED CORROSION [mm/yr.]
chemical process	- 982	9 x 10^7	3,96 x 10^{-4}	0,0059
electrochemical process	- 698	8 x 10^7	4,48 x 10^{-4}	0,0067

CONCLUSIONS

The results of the chloride ions effects that was present in concentration range between 0.02 g/L and 3.03 g/L showed that increasing concentration of chloride ions in aqueous solutions caused the increase in the corrosion rate and therefore a decrease of lifetime of the protected structures.

The results showing corrosion resistance of various thicknesses of the galvanic zinc coatings of about 25 μm up to about 40 μm, showed the direct correlation between the increased thickness of the zinc coating and the reduction of corrosion rate. It is also seen that the corrosion potential of thinner zinc layers show trends toward the more negative values, summarizing that the lower the thickness of zinc layers, the higher their corrosion tendency. Therefore, increasing coating thickness leads to a longer lifetime of the protected structure.

The results of the corrosion resistance test, chemical and electrochemical coating process, showed that the rate of galvanic corrosion of zinc deposited to about 40 μm

thickness approximately equal to the rate of chemical coating process corrosion of zinc deposited about 100 μm thickness. We showed the positive potential of the galvanic zinc corrosion, reciprocally equals its low galvanic corrosion tendency.

Based on the results presented in this work, we conclude that the zinc coating produced in a galvanic process is much more compact and resistant structure than that achived in the hot-dipped zincing procedure.

REFERENCES

Dobovišek, Ž., (1968). *Primjena galvanskih i hemijskih prevlaka u savremenom mašinstvu*, Zavod za izdavanje udžbenika, Sarajevo.

Ferenc, S., Lajoš, H. (1980). *Korozija metala*, Tehnička knjiga, Beograd.

Filipović, I., Sabioncello, P., (1960). *Laboratorijski priručnik*, Tehnička knjiga, Zagreb.

Jelic, V., (2010). *Korozija i zaštita materijala*, PFST, Split

Juraga, I, Alar, V., Šimunović, V. Stojanović, I. (2007). *Korozija i metode zaštite od korozije*, Fakultet strojarstva i brodogradnje, Zagreb.

Martinez, S., (2007). *Elektrokemija – Elektrokemijske mjerne tehnike*, Fakultet kemijskog inženjerstva i tehnologije, Zagreb.

Martinez, S., (2007). *Elektrokemijska korozija materijala – Tehnike zaštite od korozije*, FKIT, Zagreb.

Potter, E.C., (1968). *Elektrokemija*. Školska knjiga, Zagreb.

Šušić, M. V., (1980). *Osnovi elektrohemije i elektrohemijske analize*, Naučna knjiga, Beograd.

Summary/Sažetak

Korozija je danas jedan od važnih uzročnika svjetske krize materijala i energije i uzrok je znatnih gubitaka u privredi svake zemlje. Izvjesne mjere se mogu preduzeti da se poblem minimizira, što je prvenstveno i cilj ovog rada.

Ciljevi ovog istraživačkog rada bili su da se ispita uticaj hloridnih jona na galvansku pevlaku cinka, praćenjem osobina (korozione otpornosti) galvanske prevlake cinka u rastvorima hlorida različitih koncentracija, uticaj hloridnih jona na različite debljine prevlake, galvanski nanešenog cinka, praćenjem osobina (korozione otpornosti) različitih debljina galvanski nanešenog cinka u rastvoru hlorida i uticaja hloridnih jona na cinkovu prevlaku dobijenu različitim tehnologijama, topli i galvanski postupak nanošenja cinka, praćenjem osobina (korozione otpornosti) tople i galvanske prevlake cinka u rastvoru hlorida.

Sva ispitivanja su vršena na potenciostat-u/galvanostat-u Model 263A, vođena potenciodinamičkom polarizacionom metodom i linearnom polarizacionom metodom.

Na osnovu dobijenih rezultata, prezentiranih ovim istrazivačkim radom, došlo se do zaključka da je efektivna koroziona zaštita skupa, ali nikakva zaštita je neuporedivo skuplja.

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2015

44

Print ISSN: 0367-4444

Online ISSN: 2232-7266

UDC: _____

Original Scientific Article

39-42

Validation of method for the determination of mercury in the auxiliary substances azorubine 21% and azorubine 85% using cold-vapor atomic absorption spectrometry

Petinac, S.[a,b], Mulaosmanović, E.[a], Mahmutović, O.[c], Omerović, S.[a], Džudžević Čančar, H. *[d]

[a]*Bosnalijek, Pharmaceutical and Chemical Industry, Joint Stock Company,, Sarajevo, Bosnia and Herzegovina*

[b]*Faculty of Natural and Mathematical Sciences, University of Sarajevo, Sarajevo, Bosnia and Herzegovina*

[c]*Faculty of Educational Sciences, University of Sarajevo, Sarajevo, Bosnia and Herzegovina*

[d]*Faculty of Pharmacy, University of Sarajevo, Sarajevo, Bosnia and Herzegovina*

Article info
Received: 3/11/2014
Accepted:8/6/2015

Keywords:
Azorubine,
Hg,
CV-AAS,
microwave digestion.

***Corresponding author:**
E-mail:Sanel.P@bosnalijek.ba
sanelp@outlook.com
Tel: 061/813-410

Abstract: Heavy metals, such as mercury (Hg), sometimes can be found in auxiliary substances intended for pharmaceuticals use. Although the concentration of those elements is very low, their control is very important because of its toxicity. Permissible concentration of mercury (Hg) in Azorubine 21% and Azorubine 85% is prescribed by the Directive of the European Commission concerning the specific purity criteria on food coloring. The focus of this paper is on validating reliable methods of Hg determination in auxiliary substances mentioned above, by Cold-vapor Atomic Absorption Spectrometry after microwave acid digestion of solid samples. To obtain possibly present Hg in Azorubine by conversion to Hg^{2+} ions, samples were treated with a mixture of 1 mL MQ water + 1 mL 65% HNO_3 + 1 mL 70% $HClO_4$ + 5 mL 96% H_2SO_4 and heated by microwave for 30 min. on 1000 W in sealed TFM^{TM} – PTFE tubes. The resulting solutions are diluted and analyzed for Hg using cold vapor atomic absorption spectrometry with sodium borohydride as a reducing agent. The method was successfully validated and can be applied for the determination of Hg in solid samples of Azorubine 21% and Azorubine 85%, with value of recovery factor of 95% to 104% and 96% to 105%, respectively.

INTRODUCTION

Azorubine is synthesized red color belonging to mono azo class from the group of organic azo dyes and is well soluble in water. It is used in the pharmaceutical industry and for the the food coloring, especially for the food that has to be heat treated after fermentation. (EFSA (2009).

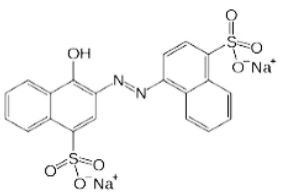


Figure 1: Azorubine (Structural formula)

Heavy metals, such as Mercury (Hg) can be found sometimes in excipients Azorubine 21% and Azorubine 85%, as a result of manufacturing process.

Like other heavy metals, mercury performs no function in the body. Mercury in its different forms is poisonous, and can be inhaled, ingested and even absorbed through unbroken skin. It is bio-accumulated and bio-magnified. Some organometallic mercury species, such as monomethyl- and dimethyl mercury are known to be extremely toxic. Although the concentration of those elements in azorubine mixture is very low, testing for the mercury is very important because of its toxicity.

Pharmaceutical companies use Azorubine in production process as base color for some syrups and solutions. The quality of these auxiliary substances is controlled prior to usage.

Permissible concentration of mercury (Hg) in Azorubine 21% and Azorubine 85% is prescribed by the Directive of the European Commission concerning the specific purity criteria on food coloring and is less than 1 part per million (0.0001 %) (EU Commission, 1999; WHO, 2008).

For the determination of trace element quantities in natural and synthesized products, the material has to be digested in a first step

Commonly used methods for digestion of complex samples involve heating samples with strong oxidation acid such is HNO_3, $HClO_4$, and H_2SO_4.

Usual methods for analyzing carbon based raw material such as coal, petroleum distillates and refined carbon based products have employed microwave assisted digest practices with various detection techniques.

The use of microwave ovens as heat sources not only drastically reduces the heating times of organic reactions, but the reactions often proceed more efficiently and selectively than when conduction heating methods are used. This is because microwave energy is transferred uniformly and almost simultaneously to the entire sample, thus eliminating any hot spots that may result in side reactions (Gilbert, Martin, 2011).

From experimental and literature source we find that best results in measuring concentration of mercury were achieved after using microwave acid digestion by mixing the test samples with 1 mL MQ water + 1 mL 65% HNO_3 + 1 mL 70% $HClO_4$ + 5 mL 96% H_2SO_4 and digesting it for 30 min. on 1000 W.

The most common analytical technique for Hg determination is cold vapor atomic absorption spectrometry (CVAAS). The CVAAS was adopted as a standard method for analysis of Hg in foodstuffs. This technique is based on the chemical reduction of mercury, usually by Sn^{2+} or BH_4^- ions to elemental Hg which is swept from the solution by a carrier gas to a quartz cell placed in the optical path of an atomic absorption spectrophotometer where the absorption of Hg is measured (Silva, Toth, Rangel, 2006).

However, no acceptable validated method for determining the mercury content in Azorubine used in pharmaceutical industry has been published so far in relevant pharmaceutical regulations, therefore there is a need for this method's validation.

The aim of the present study was to validate methods of Hg determination in Azorubine 21% and Azorubine 85%, by Cold-Vapor Atomic Absorption Spectrometry after microwave acid digestion of solid samples.

For validation process following parameters will be considered: specificity, linearity, range, accuracy, precision, detection limit, quantitation limit and stability.

EXPERIMENTAL

Material, chemicals and reagents

Following materials chemicals, and reagents were used: Azorubin – Carmoisin general formula $C_{20}H_{12}N_2Na_2O_7S_2$, known also as E122, (Frutarom Etol d.o.o. - Azorubin 21%; BTC Europe GmbH (BASF SE) – Azorubin 85%;); Mercury standard solution 1000 mg L^{-1} (Merck KgaA, Germany); 65% HNO_3 (Merck KgaA, Germany); 70% $HClO_4$ (Merck KgaA, Germany); 96% H_2SO_4 (Sigma Aldrich, England); 30% HCl (Sigma Aldrich, England); NaOH (Merck KgaA, Germany); $NaBH_4$ (Merck KgaA, Germany); 99,999 % Argon (Messer, BiH); Water quality of Milli-Q (MQ) was used in the preparation of all solutions.

The calibration curves (6-12 µg/L) for Hg were established with solutions prepared from a 1000 g/L certified stock solution.

The reducing reagent was prepared dissolving 7.5 g $NaBH_4$ and 2.5 g NaOH in 250 mL of MQ water.

As a carrier solution used 5 mol/L HCl prepared diluting 264 mL of 30% HCl to 500 mL with MQ water.

Apparatus and equipment

Microwave- assisted digestion was carried out on an instrument Microwave PRO from Anton Paar manufacturer. Mercury was determined by cold vapor technique using VGA 77 chemical vapor generation system (Varian, USA), coupled to the AA spectrometer model AA240FS (AAS Varian, USA). An Hg hollow cathode lamp (Varian, USA) operated at 6mA was used.

Procedure

A microwave assisted digestion procedure was carried out to obtain total Hg from Azorubine samples. Two replicates of Azorubine 21% and 85%, each were directly weighed (~0.50 g) into TFM™ – PTFE tubes. In each tube 1 mL of MQ water, dissolved sample and 1 mL solution of concentrated 65% HNO_3 + 1 mL 70% $HClO_4$ + 5 mL 96% H_2SO_4 was mixed together Microwave digestion was performed using a three- step program: 5 min at 1000 W power ramp, 30 min at 1000 W power hold followed by 15 min of cooling.

The resulting solution was diluted to 50 mL with MQ water. An aliquot of solution was reduced with a 3% $NaBH_4$ (w/v) in 1% NaOH and 5 mol/L HCl (as a carrier solution) and then passed to a gas-liquid separator where the evolving Hg^0 was swept in a stream of argon to the flow-through mercury cell and the atomic absorbance signal was recorded.

Measurements were carried out at the wavelength of 253.7 nm.

Concentration of mercury in Azorubine 21% and Azorubine 85% was calculated according to the following exation:

$$\frac{C \cdot 50}{m} = ppm\ Hg$$

C - measured concentration of Hg (ppb)

m- weight (mg)

RESULTS AND DISCUSSION

The values of the following parameters were determined during the analytical validation procedure: selectivity, linearity, *LOD*, *LOQ*, range, repeatability (precision), accuracy and stability (Ermer, Miller, 2005; Brentall, Clarke, 2001).

Selectivity

In the cold vapor technique, mercury is released from the sample, and then, after reduced to atomic mercury carried in a stream carrier gas to the absorption cell, where the absorption of the radiation (λ=253.7 nm) emitted by a hollow mercury cathode lamp, is measured. This measurement method guarantees high selectivity of mercury determination for two reasons: gas-liquid separator separates evolving Hg^0 gas from liquid and absorption is measured using a characteristic wavelength for mercury.

Linearity

A series of 5 standard solutions and 5 spiked sample solutions was prepared with a mercury content of 4, 6, 8, 10 and 12 ng/mL. For each of the solutions, three measurements were obtained. The calibration was carried out as a function of instrument signal and mercury content. Peak height of <1 ng Hg concentration was used as the instrument signal. Based on the results, regression parameters were found and the calibration curve determined. A high regression coefficient r (0.9987), demonstrates a high linear procedure.

Limit of Detection (*LOD*) and Quantification (*LOQ*)

The detection and quantitation limits were calculated by ratio SE/S: LOD = 3x (SE/S) and LOQ = 10x (SE/S). SE is standard error of the intercept deviation for 10 measurements of the spiked sample solutions with mercury concentration in expected LOQ range and S is the slope of the calibration graph correspond to Hg concentration.

LOD was taken to be 0.9075 ng/mL. Calculated for a mass of a sample of 500 mg, this corresponds to Hg concentration in Azorubine samples of 90.7 ng/g. However, LOQ was determined to be LOQ=3.3·LOD, i.e. 2.99 ng/mL (299 ng/g).

Range

The measurement range is a concentration range from the LOQ section to the maximum standard solution concentration used for calibration; it is therefore equal to 3.00-12.00 ng. Calculating for the mass of the sample determined to be 500 mg, this corresponds to a mercury concentration range of 300 ng/g-1200 ng/g.

Repeatability (precision)

Repeatability was determined from a series of six independent measurements of standard solutions of mercury in target concentration (10 ppb) and real samples with added mercury in same target levels. It was determined as the CV value for the series.

Obtained results (Table 1. and Table 2.) demonstrate a high level of repeatability (precision).

Accuracy

Repeatability was determined from a series of three independent measurements for each of three spiked sample solutions with mercury concentration of 80%, 100% and 120% from target concentration (10 ppb). It was determined as the Recovery value for the series.

From obtained results (Table 1. and Table 2.) it can be concluded that used method is very precise.

Stability

Stability test was conducted by measuring three standard solutions in 3 different concentrations (80%, 100% and 120%) and real samples with added mercury of the same concentration, freshly prepared and after 24 hours at ambient conditions.

As an indication of the stability of the solution, % Difference (%D) was calculated according to the equation:

$$\%D = \frac{[c(found\ (fresh)) - c(found\ (after\ 24\ hours))] \cdot 100}{c(found(fresh))}$$

Obtained results (Table 1. and Table 2.) demonstrate that solutions of standard and samples were stable for 24 hours at ambient conditions.

Table 1: Results of validating methods of Hg determination in auxiliary substances Azorubine 21% by Cold-Vapor Atomic Absorption Spectrometry after microwave acid digestion

Tested parameters	Results	Acceptance criteria
Specificity	No interference	No interference
Accuracy (Recovery)	95.07% ÷ 104.30%	80% ÷ 120%
Detection limit	0.9075 μg mL^{-1}	-
Quantification limit	2.9947 μg mL^{-1}	≤ 0,5x limit
Repeatability (CV)	0,2489%	≤ ± 5%
Intermediate precision (CV)	3.01%	≤ ± 20%
Linearity	y = 0.015 + 0.0033x	
Correlation coefficient	R = 0.9987	0.99
Stability (%D)	0,52%	< 20,0%

Table 2: Results of validating methods of Hg determination in auxiliary substances Azorubine 85% by Cold-Vapor Atomic Absorption Spectrometry after microwave acid digestion

Tested parameters	Results	Acceptance criteria
Specificity	No interference	No interference
Accuracy (Recovery)	96.46% -105.50%	80% - 120%
Detection limit	0.9075 μg mL-1	-
Quantification limit	2.9947 μg mL-1	≤ 0,5x limit
Repeatability (CV)	0,9942%	≤ ± 5%
Intermediate precision (CV)	2.16%	≤ ± 20%
Linearity	y = 0.015 + 0.0033x	
Correlation coefficient	R = 0.9987	0.99
Stability (%D)	-0,30%	< 20,0%

CONCLUSION

Based on the results, obtained from validating methods of Hg determination by Cold-Vapor Atomic Absorption Spectrometry after microwave acid digestion, it can be concluded:

1. All the validation results are in accordance with acceptance criteria.

2. The method was successfully validated and can be applied for the determination of Hg in solid samples of Azorubine 21% and Azorubine 85% with recovery factor value of 95% to 104% and 96% to 105%, respectively.

ACKNOWLEDGMENTS

This project was supported by the Bosnalijek, Pharmaceutical and Chemical Industry, Joint Stock Company.

REFERENCES

Brentall, A.E., Clarke, G.S. (2001) Validation of analytical methods. In Ahuja, S., Scypinski, S. (Ed.), *Handbook of Modern Pharmaceutical Analysis.* (p.p. 429-457). Academic Pres.

EFSA (European Food Safety Authority) (2009). *Scientific Opinion on the re-evaluation of Azorubine/Carmoisine (E 122) as a food additive.* Parma, Italy.

Ermer, J., Miller, J.H.M. (2005). *Method Validation in Pharmaceutical Analysis*. (1st Ed.) WILEY-VCH Verlag GmbH & Co. KGaA.

EU Commission (1999). *Specific purity criteria concerning colours for use in foodstuffs*. Commission Directive 1999/75/EC.

Gilbert, J., Martin F.S. (2011*). Experimental Organic Chemistry- A Miniscale and Microscale Approach*. (5th Ed.) BROOKS/COLE Cengage Learning.

Silva, M.F., Toth, I.V., Rangel, A.O. (2006). Determination of mercury in fish by cold vapor atomic absorption spectrophotometry using a multicommuted flow injection analysis system. *Analytical Sciences*, *22 (6)*, 861–864.

WHO - Word Health Organization (2008). *Development of Paediatric Medicines:Pharmaceutucal Development*. WHO QAS/08.257.

Summary/Sažetak

Teški metali, kao što je živa (Hg), ponekad se mogu naći u polaznim supstancama za farmaceutsku upotrebu. Iako je njihova koncentracijaveoma niska, praćenje istih je veoma važno zbog njihove toksičnosti. Dozvoljena koncentracija Hg u Azorubinu 21% i Azorubinu 85% je propisana Direktivom Europske komisije u vezi s posebnim kriterijima čistoće boja za hranu. Fokus ovog rada je na validaciji precizne i tačne metode određivanja Hg u Azorubinu 21% i Azorubinu 85%, koristeći tehniku hladnih para atomske apsorpcione spektrometrije nakon mikrotalasne kiselinske digestije čvrstih uzoraka. Da bi eventalno prisutnu Hg u Azorubin 21% i Azorubinu 85% preveli u obliku Hg^{2+} iona uzorci su tretirani smjesom koja sadrži 1 mL MQ vode + 1 mL 65% HNO_3 + 1 mL 70% $HClO_4$ + 5 mL 96% H_2SO_4 i zagrijavani mikrovalnim zračenjem u periodu od 30 min. na 1000 W. Koncentracija Hg u uzorku je nakon toga određena tehnikom hladnih para atomske apsorpcione spektrometrije uz natrij borohidrid kao rededucens. Metoda je uspješno validirana i može se primjenjivati za određivanje Hg u čvrstom uzorku Azorubina 21% i Azorubina 85%, uz vrijednost *recovery* faktora od 95% -104% i 96-105%, redom.

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2015

44

Print ISSN: 0367-4444
Online ISSN: 2232-7266

UDC: _____

Original Scientific Article

43-52

Antioxidant and prooxidant activities of phenolic compounds of the extracts of *Echinacea purpurea* (L.)

Jukić, H.[a], Habeš, S.[b], Aldžić, A.[a] , Durgo, K.[c], Kosalec, I.[d]

[a] *University of Bihac, College of Medical Studies, Žegarska aleja bb, 77000 Bihac, Bosnia and Herzegovina*

[b] *University of Sarajevo, Faculty Of Health Studies Sarajevo, Bolnička 25 71000 Sarajevo, Bosnia and Herzegovina*

[c] *University of Zagreb, Faculty of Food Technology and Biotechnology, Pierottijeva 6, 1000 Zagreb, Croatia*

[d] *University of Zagreb, University of Zagreb, Faculty of Pharmacy and Biochemistry, Ante Kovačića 1, 10000 Zagreb, Croatia*

Article info
Received: 11/5/2015
Accepted:8/6/2015

Keywords:
antioxidants,
cytotoxicity,
poliphenolics,
Echinacea purpurea

***Corresponding author:**
Huska Jukić,
Phone: +38761751969
Phone/fax: +38737227046
E-mail: huskaj037@gmail.com

Abstract: Background and objectives: In recent years, there is a *growing interest* on natural and safer *antioxidants*. So far, little is known about the cytotoxic and (anti) oxidative potential of echinacea (*Echinacea purpurea*) extracts.

Methods: In order to evaluate the antioxidant activity of extracts, total phenolics content and the scavenging capacity on DPPH˙ radicals was determined. The ability of extracts to scavenge superoxide and hydroxyl radicals was tested using electron paramagnetic resonance (EPR) techniques. Also, the extracts were screened for cytotoxicity and antioxidative/prooxidative potential by neutral red and DCFDA assay respectively, using human colon cancer cell line SW480. *The cells were* exposed to *various concentrations* of extracts (range: 0,008; 0,08; 0,8; 1; 10 i 20 mg/mL) and *different treatment* times (2, 3, 4 and 24 h).

Results: The content of total phenolic compounds of extracts of *E. purpurea* was 10.57 % GAE. The scavenging activity of radicals was found to exhibit 50% of the inhibition value (IC50 value) at the concentration of 15.67 μg/ml for the investigated echinacea extract. Also, the calculated value of 210 mg/ml for hydroxyl and 76.7 mg/ml for superoxide anion radical indicates that the Echinacea extract is rich in antioxidant compounds that neutralize investigated radical species. In *in vitro* experiments, echinacea extract showed prooxidant effect at lower concentrations and shorter incubation period when SW480 cell line was used as test system. The highest concentration was also the most toxic which is particularly evident after 24 hours of treatment.

Conclusions: Echinacea extracts are shown to possess the strong antioxidant potential.

INTRODUCTION

Phenolic compounds are widely present *in almost all plants* and *food* products of *plant origin*. Polyphenols in plants can act as signaling molecules involved in the hormonal regulation of plant growth, and protect them from infection by microorganisms (antimicrobial activity). They also act as protective agents against UV radiation, attract pollinators and contributors in plants pigmentation (Naczk et al., 2004). In food, *phenolic compounds* contribute bitterness, clarity, colour, taste, flavour and oxidative stability. With multiple biological activities, extracts rich in phenols are of particular importance for the food industry because they slow down the oxidative degradation of lipids and thus improve the quality and

nutritional value of food (Mathew et al., 2006.). Also, consumption of phenols from plant based foods is associated with reduced risk of a variety of diseases.
In recent years, a growing interest in the food industry for the use of antioxidants from natural sources (e.g., polyphenolic compounds from plants, fruits and vegetables, whole grains) has been noticed. There is a growing body of evidence that the most commonly used synthetic antioxidants have potential to damage health. Food producers, in order to protect consumers, their interests and their safety have drawn attention to production of natural antioxidants instead to synthetic one. Benefits of polyphenols intake are result of their antioxidant activity, the presence in the human diet and their influence in the prevention of various chronic diseases associated with oxidative stress (Zhou et al., 2006.; Vinson et al., 1998.; Teow et al., 2007.). In our research we were investigated chemical composition and antioxidant activity of *Echinacea purpurea* (L.) extracts (Echinacea extracts). *Echinacea purpurea* (L.) is a good example of species that contains a number of bioactive compounds with potential antioxidant properties. So far, little is known about the potential of those extracts.

MATERIALS AND METHODS

Chemicals

Ethanol, ethylenediaminetetraacetic acid (EDTA), pyrogallol, tannic acid (95%) were purchased from Kemika (Zagreb, Croatia). Chlorogenic acid, 2,2-diphenyl-1-picryl-hydrazyl (DPPH$^{\bullet}$), rosmarinic acid (96%), 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), dimethylsulfoxide (DMSO) and sodium molybdate were obtained from Sigma-Aldrich (St. Louis, MO, USA). Butylated hydroxytoluene (BHT, ≥99%) and quercetin-3-rutinoside (rutin, ≥95%) were obtained from Fluka (Buchs, Switzerland). Folin–Ciocalteu's phenol reagent, 3-*tert*-butyl-4-hydroxyanisole (BHA) and were obtained from Merck (Darmstadt, Germany). Dulbecco's modified Eagle medium (DMEM), Fetal bovine serum (FBS) and antibiotics were obtained from (GIBCO, Grand Island, NY, USA). All used chemicals and reagents were of the highest analytical grade and obtained from "Kemika" Zagreb (Croatia).

Plant material

We used air dried aerial parts of purple Echinacea, taken from the Jan-Spider (Pitomača, HR). Samples were collected during the year of 2010.

Sample preparation

Sample preparation for antioxidant analysis

In the previously milled plant material (20.00 g) was added 200 mL of 70% ethanol and left to stand overnight at room temperature. Extraction of the ethanol dissolving compounds was then continued by applying ultrasound (30 min), using the ultrasonic bathroom Branson model b-220 Smith Kline Co., Shelton, USA (50/60 Hz, 125 W). The sample was filtrated and in plant material was added the new amount of 200 mL of 70% ethanol, and then previously described procedure of ultrasonic extraction was repeated. After filtration, the obtained filtrate by the first and second filtration were combined and dried by evaporation under vacuum at 313 K.

Sample preparation for electron paramagnetic resonance (EPR) techniques and for determining prooxidant and antioxidant effects of extracts on human cell line of colon carcinoma (SW 480)

The sample was milled for further analysis. After the addition of 200 mL distilled water to 20 g of sample, extraction of the water dissolving compounds was performed by ultrasound (30 min) at 303 K, using the ultrasonic bathroom Branson model b-220 Smith Kline Co., Shelton, USA (50/60 Hz, 125 W). The water extract were filtrated and dried by lyophilization. The obtain extract was used for electron paramagnetic resonance (EPR) techniques and to determine prooxidant and antioxidant effects of extracts on human colon carcinoma cell line (SW 480).

Determination of polyphenols

Determination of total polyphenols of the overground parts of the tested plant species was carried out by spectrophotometric method according to the method by Schneider (Schneider, 1976). Blue solution absorbance was measured at 720 nm with distilled water as a blank test. To calculate the concentration of total polyphenols, calibration line was prepared. For this purpose 10 mg of tannic acid was dried at 80 °C and dissolved in 100 mL of distilled water. Blue solution absorbance was measured at 720 nm with distilled water as a blank test. From the linear equation ($y = 9.7768\ x + 0.0099$) the proportion of polyphenols is calculated by first calculating the value of x corresponding to mg of polyphenols in the sample which is according to the procedure diluted 1,000 times. Then the polyphenols on the amount of plant material taken into the procedure are calculated (0.25 g) and finally expressed as a percentage. Share of polyphenols (%) = $x \bullet 100 / m$, x = value in mg (calculated from the calibration line) x 10-3, m = plant material in g.

Determination of total flavonoids

The total flavonoid contents of tested plant extract were determined using the spectrophotometric method of Christ et al., (1960). Briefly, each powdered plant sample (0.2 g) was mixed with 20 mL of acetone, 2 mL of 25% hydrochloric acid and 1 mL of 0.5% hexamethylenetetramine solution and heated under reflux in a water bath for 30 min. The extract was filtered and re-extracted twice with 20 mL of acetone for 10 min. Filtrates were combined and made up to 100.0 mL with acetone. An aliquot of 20 mL of the acetone extract was mixed with 20 mL of water and then extracted with three quantities, each of 15 mL, of ethyl acetate. Combined ethyl acetate layers were washed twice with water then filtered and diluted to 50.0 mL. To 10.0 mL of this solution 0.5 mL of 0.5% solution of sodium citrate and 2 mL of 2% aluminium chloride solution (in 5% methanolic solution of acetic acid) was added and then diluted to 25.0 mL with 5% methanolic solution of acetic acid. A sample solution prepared in the same manner but without addition of aluminium chloride solution served as a blank. All determinations were performed in triplicate. The percentage content of

flavonoids, expressed as quercetin, was calculated from the equation: *(%) = A × 0.772/b*, where *A* is the absorbance of the test solution at 425 nm and *b* is the mass of the sample, in grams.

Antioxidant activity of *E. purpurea* extract

2, 2-Diphenyl-1-picrylhydrazyl radical (DPPH•) radical scavenging assay

The free radical scavenging activities of the samples were measured using the stable DPPH• radical, according to the method of Blois (1958). Briefly, 0.1 mM solution of DPPH• in ethanol was prepared and 1 mL of this solution was added to 3 mL of sample solution in ethanol at different concentrations (0.39-200 μg/mL). The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm. The capability to scavenge the DPPH• radical was calculated using the following equation: $(\%) = [(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of sample, corrected for the absorbance of sample itself. Butylated hydroxytoluene (BHT) was used for comparison. All determinations were done in triplicate.

ESR measurements

Hydroxyl radical scavenging activity

The influence of *E. purpurea* extract on the formation and stabilization of hydroxyl radicals was determined by adding investigated extracts in the Fenton reaction system at the range of concentrations 25-1500 μg/ml. Hydroxyl radicals are identified because of their ability to form nitroxide adducts (stable free radicals form) from the commonly used DMPO as the spin trap (Buettner, 1985). The Fenton reaction was conducted by mixing 200 μl of DMPO (112mM), 200 μL of DMF, 200 μL of H_2O_2 (2mM) and 200 μL of $FeCl_2$ (0.3 mM) (control). ESR spectra were recorded after 5 minutes, with the following spectrometer settings: field modulation 100 kHz, modulation amplitude 0.226 G, receiver gain 5 x10^5, time constant 80.72 ms, conversion time 327.68 ms, center field 3,440.00 G, sweep width 100.00 G, x-band frequency 9.64 GHz, power 20 mW, temperature 23°C. The SA_{OH} value of the extract was defined as: $SA^{\bullet}_{OH} = 100 \times (h_0 - h_x) / h_0$ [%]; where h_0 and h_x are the hight of the second peak in the ESR spectrum of DMPO-OH spin adduct of the control and the probe, respectively.

Superoxide anion radical scavenging activity

Superoxide anion radicals ($O_2^{\bullet-}$) were generated in the reaction system obtained by mixing 500 μL of dry dimethylsulfoxide (DMSO), 5 μL of KO_2 / crown ether (10 mM / 20 mM) prepared in dry DMSO and 5 μL of 2 M DMSO solution of DMPO as spin trap. The influence of extracts on the formation and transformation of superoxide anion radicals was obtained by adding the DMF solution of *E. purpurea* extract to the superoxide anion reaction system at the range of concentrations 5-100 μg/ml. After that the mixture was stirred for 2 min and transferred to a quartz flat cell ER-160FT. The ESR spectra were recorded on an EMX spectrometer from Bruker (Rheinstetten, Germany) under the following conditions: field modulation 100 kHz, modulation amplitude 4.00 G, receiver gain 1 × 10^4, time constant 327.68 ms, conversion time 40.96 ms, center field 3440.00 G, sweep width 100.00 G, x-band frequency 9.64 GHz, power 20 mW, temperature 23 °C. The $SAo_2^{\bullet-}$ value of the extract was defined as: $SAo_2^{\bullet-} = 100 \times (h_0 - h_x) / h_0$ [%]; where h_0 and h_x are the height of the second peak in the ESR spectrum of DMPO-OOH spin adduct of the control and the probe, respectively.

Cell line

Human colon cancer cell line (SW 480) was used in all *in vitro* experiments. The cell line was obtained from the Ruđer Bošković Institute, Zagreb. Monolayer culture of human cells grown in DMEM medium with 10% calf serum at 37 °C in a humid atmosphere with 5% CO_2 was used. During the transplant of the cells, 0.25% solution of trypsin was used for detachment of cell monolayers.

Determination of prooxidant and antioxidant effects of the echinacea extracts on human colon carcinoma cell line (SW 480)

Non-toxic concentrations of hydrogen peroxide is used, which will cause oxidative stress in the cells. This is done in a way that 10 mL 6% H_2O_2 is added to 990 mL of PBS. In this way, a solution which will be used to treat cells is obtained, 0.06% concentrations of H_2O_2 (conc. 17.6 mM). After trypsinization and cell counting, cell suspension is prepared at a concentration of 105 cells/mL and 180 mL of cell suspension is inoculated in a black microtiter plate with 96 wells. The next day, after the cells tied to the bottom of the wells, the cells are treated for half an hour with 100 mL of 0.06%-percent H_2O_2. Thereafter, hydrogen peroxide is removed, and various concentrations of plant extracts (0.008, 0.08, 0.8, 1, 10 and 20 mg, / ml) are placed on the cells for 2, 3, 4 and 24 hours with and without recovery. After treatment, the medium is removed and cells are washed with PBS. Solution of DCFH-DA is prepared in PBS containing 1% bovine serum albumin (BSA) and the cells are treated with solution of DCFH-DA for half an hour. After that the intensity of fluorescence is determined in the fluorimeter at excitation wavelength of 485 nm and emission wavelength of 520 nm. To express the measured fluorescence as the percentage of surviving cells, neutral red test is performed again as well as calculation of the quotient of survival. Results are expressed as the ratio of fluorescence and survival quotient which is calculated relative to the negative control. Each experiment was repeated three times, and each concentration was tested in triplicate.

$$\text{DCF} = \frac{\text{fluorescence (arbitrary unit)}}{Q_{survival}}$$

Negative controls show the basic level of ROS measured in the cells that are not treated, and positive control represents the level of ROS in the cells treated with hydrogen peroxide.

''Neutral red'' test

Treatment of cells was performed during the exponential phase of cell growth at 2, 3, 4 and 24 hours, corresponding to the exposure of cells of the investigated compounds during one cell division. Therefore, 105 cells / mL was seeded in the 96-well plate. The next day, after the cells

had adhered to the surface and after the division started, cells were treated with plant extracts at different concentrations (0.008 , 0.08, 0.8 , 1, 10 and 20 mg/mL). As a control, cells grown in growth medium were used. After the treatment, medium was removed and cells were washed for twice with 100 μL PBS. The "neutral red" solution was added in the wells and cellswere incubated for 90 min at 37°C. "Neutral red" was then removed and the cells were again washed twice with 100 μL of PBS. Color remains accumulated in the lysosomes of viable cells. In order to extract the color, 100 μL of mixture of ethanol/water/glacial acetic acid (50:49/1) was added on the washed cells (Costa et al., 2005). The intensity of color separation was determined spectrophotometrically at 430 nm. Dead or damaged cells do not retain their color after washing and fixation processes (Babich et al., 1990.).
Percentage of survival is determined in relation to the negative control by the formula set out below: % Survival = (A430 nm of the studied compound / A430 nm of control) x 100 Each experiment was repeated 3 times, and each concentration was investigated in 4 replicas.

Statistical Analysis

One-way analysis of variance (ANOVA) and multiple comparisons (Duncan's *post-hoc* test) were used to evaluate the significant difference of the data at $p < 0.05$. All experiments were performed at least in triplicate. Results are presented as mean values ± SD.

RESULTS AND DISCUSSION

The total amount of polyphenols in Echinacea extracts
The content of total phenolics compounds in plant material was (10,57 ± 0,35)%, expressed as g of gallic acid per 100 g of the dry sample (%; w/w). Total flavonoids content was (0,13 ± 0,004)%, expressed as g of quercetin per 100 g of the sample.
The reviewed scientific literature states that large differences in the values of total polyphenols can be observed, which are determined by spectrophotometry, with appliance of the Folin-Ciocalteuova reagent. The method according to the Folin-Ciocalteu is fast and widely used, however, it is not quite specific and it can detect phenols with different sensitivity (Kahkonen et al., 1999.). Hence, the results do not provide a complete quantitative and qualitative picture of the polyphenolic compounds in the extracts, due to the possible presence of interfering compounds (Singleton et al., 1999.). A number of environmental factors that contribute to the variability of polyphenols in plant material should be added to the fact. The plant world recognizes more than 4,000 flavonoids, which are used in the traditional and Eastern medicine over a thousand years (Hertog et al., In 1992. Peterson et al., 1998.). Flavonoids are attributed with positive effects on human health, which are manifested through its anticarcinogenic, antibacterial, immune-stimulating, anti-virus and the anti-inflammatory properties (Havseen, 2002.). The benefit of fruits and vegetables consumption is largely attributable to the positive effects of flavonoids (Howard et al., 1997.).

Antioxidant activities of E. purpurea ethanolic extracts
Polyphenolic compounds such as flavonoids, phenolic acids and tannins are considered to be the major contributors to the antioxidant activity of medicinal plants, fruits and vegetables (Pereira et al., 2009.; Rice-Evans et al., 1996.). Therefore, in the present study five different assays were employed in order to determine and compare the antioxidant properties of selected *Echinacea* species, as well as to elucidate their mode of action. The antiradical activity of the ethanol extract of the overhead part of the species *Echinacea purpurea*, chlorogenic acid, rutin, tannic acid, was copared with the synthetic antioxidant butyl-hydroxy anisole (BHA). After measuring absorptions at 517 nm, the percentage of the inhibition capacity of DPPH$^{\bullet}$ radicals were calculated. The plant extract in lower amounts had quite a weaker effect then the synthetic antioxidant. Although it lags continually after the effect of BHA, the difference is significantly lowered in the amounts above 50 μg/ml. It was also revealed that the chlorogenic acid, rutin and tannic acid are better catchers of DPPH$^{\bullet}$ than the referent antioxidant. The effect of BHA is equalised with the effect of rutin only at the amount of 12,5 μg/ml when it accomplished the inhibition above 85%. The strongest antiradical activity was determined for the tannic acid which already in the amount of 0,78 μg/ml accomplishes a 50% exhibition of DPPH$^{\bullet}$. The chlorogenic acid shows the same effect in the amount of 1,56 μg/ml and is equalised with the tannic acid in the concentration of 6,25 μg/ml. In the Picture 1 it is visible that concentrations higher than 15 μg/ml achieve a 50% inhibition of free radicals. Concentrations higher than 50 μg/ml approaches the effect of pure substances and BHA. These results show that flavonoids, phenolic acid and tannins, present in the examined species, equally contribute to the antiradical effect of the extract. The research of Yokozawa et al., (1998.) has shown that tannins and some flavonoids show an activity in relation to DPPH$^{\bullet}$ radicals and that the activity is closely related to their chemical structure. With the increase in galiol groups, the molecular mass and ortho-hydroxy groups in the structure, the activity of tanine increases, and the number and position of hydroxyl groups represent an important characteristic of flavonoids as a "quenchers" of free radicals. Fenglin et al. (2004.) released the results of the study of the 'scavangers' activity on DPPH radicals of water-methanol extracts of more then 300 medicinal herbs. For 56 of the examined specimens they got EC_{50} values under 0,500 mg of the specimen/ml of the extragent. The same authors attribute the activity of DPPH$^{\bullet}$ radicals of plants to the present flavonoids and tannins in the extract. Chen et al., (2004.) discovered that the chlorogenic acid most actively removes DPPH$^{\bullet}$ radicals in plants, and that its activity in the same test is the same and larger than the activity of tocopherol. Orhan et al. (2009.) got similar results when they studied antioxidant activities of the species *E. purpurea* and *E. pallida* by determining the catching capacity of DPPH$^{\bullet}$ of free radicals and chelate ions of iron. A chrloroform extract in air of dry plant material *E. purpurea* showed the greatest capacity of chelate iron ions (Orhan et al., 2009.).

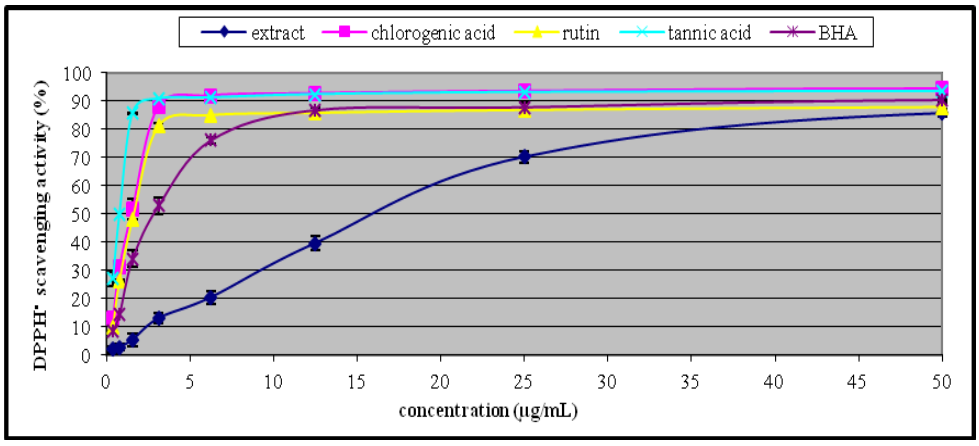


Figure 1. Antioxidant activities of *E. purpurea* ethanolic extracts

Results ESR

Other part of our investigation on antioxidant activity of *E. purpurea* extract was the measurement of the scavenging activities on hydroxyl and superoxid anion radicals by ESR method. Using a spin trap, such as DMPO, it is possible to convert reactive hydroxyl radicals to stable nitroxide radicals (DMPO-OH adducts) with spectral hyperfine splitting that reflects the nature and structure of these radicals. The reaction of Fe^{2+} with H_2O_2 in the presence of the spin trapping agent DMPO generated a 1:2:2:1 quartet of lines with hyperfine coupling parameters (aN=aH= 14.9 G) (Čanadanović-Brunet, et al., 2005.). The intensity of the ESR signal, corresponding to the concentration of free radicals formed, was decreased in the presence of different amounts of *E. purpurea* extract. The total elimination of hydroxyl radical (SA_{OH}=100%) was obtained in the presence of 1500 µg/ml of extract, which indicates that this applied concentration completely inhibits the production of hydroxyl radicals. The investigated extract showed dose-dependent radical scavenging activity. The EC_{50} value, defined as the concentration of extract required for 50% scavenging of superoxid anion radicals under experimental condition employed, is a parameter widely used to measure the free radical scavenging activity (Cuvelier et al., 1992.); a smaller EC_{50} value corresponds to a higher antioxidant activity. The EC_{50} value of *E. purpurea* extract (76.7 µg/ml) shows that extract is rich in antioxidant compounds and efficiently scavenge superoxide anion radicals.

Determination of oxidative and antioxidant effect of extracts of cultured human colon cancer cells (SW 480)

In order to determine potential antioxidant effect of Echinacea extracts against free radicals produced by hydrogen peroxide which was added onto cells, colon cancer cells (SW-480) were pretreated with hydrogen peroxide and subsequnently they were exposed to different concentrations of Echinacea extracts. Figures 2-4 show the changes made to the contents of ROS in the cells treated only with Echinacea extracts and in the cells pre-treated with H_2O_2 respectively. As it can be seen on Figure 2, there is a lower intensity of the positive control fluorescence, i.e. cells treated with a two-hour treatment with H_2O_2 in the presence of a medium (a medium in which the cells were growing and in which dilutions were made - DMEM + 10 % serum) as compared to untreated cells (negative control). In fact, the level of oxidative stress is more or less stable in the positive control cells despite various times of incubation, as opposed to the basic oxidative stress in the negative control cells, which decreases progressively (Figure 2-5). Oxidative stress in the positive control was lower as compared to the half-hour treatment with hydrogen peroxide without treatment. Obviously, treatment with H_2O_2 leads to formation of ROS 's, but also leads to the simultaneous induction of detoxifying enzymes in the cells that neutralize ROS and the volume of oxidative stress in the induced cells decreased. It is unclear why a longer incubation reduces oxidative stress in the negative controls (Figures 3 and 4), although the formation of hydrogen peroxide and rapid degradation of polyphenolic compounds in similar cell cultures has been observed (Long et al., 2010.). Addition of low concentrations (up to 0.8 mg/mL) of the Echinacea extract acted as a prooxidant after 2 h of incubation. After 3 and more hours of incubation this effect had faded, and the addition of the extract generally did not raise the amount of ROS's in comparison to the negative control (Figure 3-5). Similarly to the effect of the positive control, in the cells pretreated with hydrogen peroxide, and than with the extract, significant reduction in oxidative stress occured. Antioxidant activity was strong and generally strengthens with the amplification of concentration of added extract, regardless of the time of incubation (Figures 2-5). Only for two hours and three hours incubation, decline of oxidative stress at low concentrations of extract and increase at higher concentrations has been observed. This discrepancy could be explained by the time required for the formation of a critical amount of ROS`s due to the interaction of H_2O_2 and polyphenol extract, while longer incubation time leads to the suppression of oxidative stress by induction of cellular antioxidant protection. Prooxidant effect of many phenolic compounds was observed in diclorofluoresceine and other *in vitro* tests. For example dopamine, in concentrations of less than 500 µM, decreases the fluorescence intensity thus showing his

antioxidant properties, while at a concentration of 1 μM increases the fluorescence intensity, acting as a prooxidant (Wang and Joseph, 1999.). It was found that lower concentrations of flavonoids (nM to low values μM) can stimulate antioxidant genetic response including the detoxification enzymes of phase II. On the other hand, higher concentrations of the flavonoids may support the activation of mitogen-activated protein kinases which can lead to apoptosis (Chen et al., 2000.; Kong et al., 2000.). Many reports describe the negative effects of flavonoids on the cellular level. For example, due to the toxic effects of flavonoids at high concentrations, several studies have suggested a DNA strand breakage when flavonoids such as epigallocatechin-3-gallate are used (Tian et al., 2007.), quercetin (Beatty et al., 2000.), and kaempherol (Niering et al., 2005.). SW480 are cancer cells, and they have higher levels of ROS's, so the extracts could by prooxidant activity induce apoptosis in such an environment (Hail et al., 2008.). The results confirm the prooxidant effects of extracts at lower doses which is consistent with the hypothesis on the control of transformed cells by apoptosis induced by phytochemicals (Hail et al., 2008.). Numerous other mechanisms of anticarcinogenic polyphenols activities have been proposed, some of which are unrelated to the redox capacity (Finley, 2005.). Of course, one must not forget the fact that the tests were carried out in vitro or ex situ. To determine the full effect of the extracts and their components, it will be necessary to take into account the processes of absorption and metabolism under the action of digestive enzymes and intestinal microflora, distribution, degradation in the intestinal mucosa and liver, conjugation and excretion. Intestinal microbial enzymes hydrolyze flavonoid glycosides to their aglycone and sugar. Most aglycones are then metabolized by the microorganisms, while a negligible part is absorbed (Gugler et al., In 1975. Hollman et al., 1995.). The low doses of polyphenols are more relevant for modeling of their effect in the organism with respect to the concentration of these compounds in the plasma after the average daily intake (50 mg) ranging up to 0,4 microns, i.e., about $0{,}03 \cdot 10^{-6}$ mg/mL of quercetin (Manach et al., 2005.).

Ehinacea purpurea

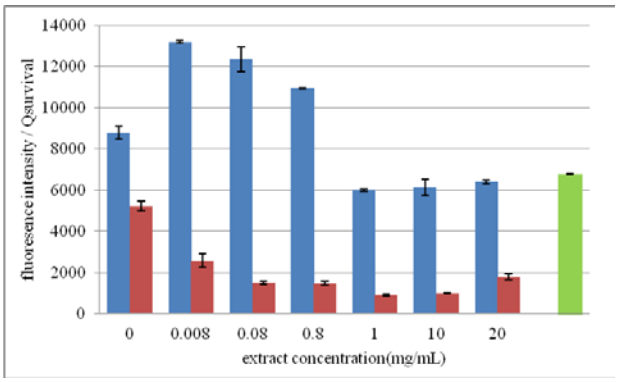


Figure 2. Oxidative stress determined by diclorofluoresceine test after treatment of SW480 cells with H_2O_2 and/or extract of purple Ehinacea after two-hour incubation

blue - cells treated with Echinacea extract (at concentrations of extract 0 only the medium was added) red - cells treated with H_2O_2 (at concentrations of extract 0 only the medium was added), green - cells exposed to a half-hour treatment with H2O2; * height of the column represents the mean of three determinations ± SD.

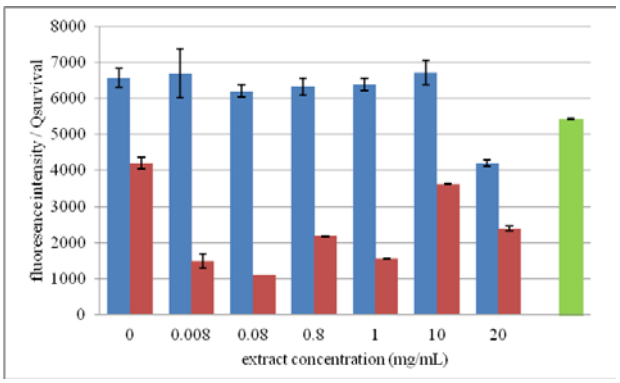


Figure 3. Oxidative stress determined by diclorofluoresceine test after treatment of SW480 cells with H_2O_2 and/or extract of *E. purpurea* after three hour incubation

blue - cells treated with Echinacea extract (at concentrations of extract 0 only the medium was added) red - cells treated with H_2O_2 (at concentrations of extract 0 only the medium was added), green - cells exposed to a half-hour treatment with H_2O_2; * height of the column represents the mean of three determinations ± SD.

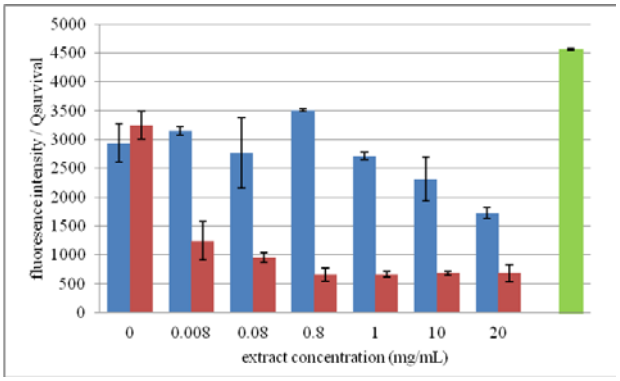


Figure 4. Oxidative stress determined by diclorofluoresceine test after treatment of SW480 cells with H_2O_2 and/or extract of *E. purpurea* after four hour of incubation

blue - cells treated with Echinacea extract (at concentrations of extract 0 only the medium was added) red - cells treated with H_2O_2 (at concentrations of extract 0 only the medium was added), green - cells exposed to a half-hour treatment with H_2O_2; * height of the column represents the mean of three determinations ± SD.

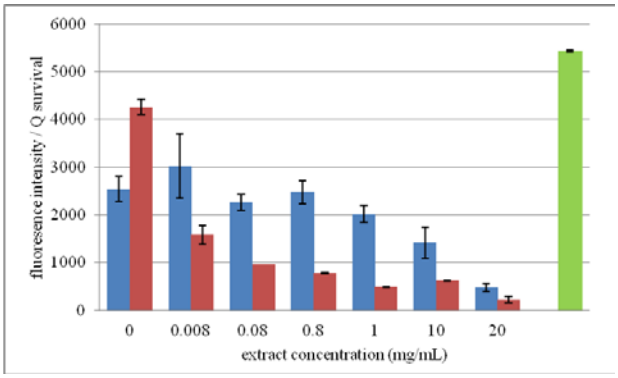


Figure 5. Oxidative stress determined by diclorofluoresceine test after treatment of SW480 cells with H_2O_2 and/or extract of *E. purpurea* with twenty four hours of incubation

blue - cells treated with Echinacea extract (at concentrations of extract 0 only the medium was added) red - cells treated with H_2O_2 (at concentrations of extract 0 only the medium was added), green - cells exposed to a half-hour treatment with H_2O_2; * height of the column represents the mean of three determinations ± SD.

Cytotoxicity Test

Determination of the cytotoxic effect of plant extracts on human colon carcinoma cells (SW 480)

Cytotoxicity of extracts of Echinacea herb was determined on a human cell line of colon cancer SW 480 in six different concentrations of the extract with different duration of treatment. Being that in the studied extracts of species *E. purpurea*, according to qualitative analysis, the presence of polyphenolic compounds that may help prevent cancer, including flavonoids, was noted, the antiproliferative effects of extracts are expected. Kuntz et al. (1999) found that polyphenols may play a significant role in preventing colon cancer by blocking the hyperproliferative epithelium by inducing apoptosis. The ability of a compound to inhibit the growth of tumor cells in a culture is an indication of its potential value as a therapeutic agent in vivo (Lieberman et al., 2001.). According to **Figure 6**, the Echinacea extract treatment for 2 and 3 hours shows a slight increase in the number of cells at the highest tested concentration. When treated for 3 and 4 hours, the extract showed no cytotoxicity effect to cells SW 480. After 24 hours of treatment a slight decrease in surviving cells as regard to concentration is observed.

Induction of apoptosis is important issue and it was suggested that the *in vitro* apoptotic activity should be used in the evaluation of potential phenolic phytochemicals in cancer prevention (Hsu et al., 2003b). Among the most important findings in the field of biology and genetics of cancer is that the genes that control apoptosis have the greatest effect on malignancy in case of disruption of their function, which can cause duplication of damaged cells, tumor initiation, progression and metastasis. Therefore, one of the mechanisms that prevent tumor production by use of natural products which are rich on different phytochemicals, could be the induction of apoptosis, which can be the basis for the prevention and therapy of cancer with biologically active compounds (Colic and Pavelic, 2000.). An increasing list of chemopreventive agents, including many food ingredients, for example, polyphenols, and synthetic derivatives, which have been shown to activate apoptosis of tumor cells *in vivo* and/or *in vitro*. Moreover, the vast majority of these agents induce mitochondrial - mediated apoptosis due to their prooxidant effects on transformed cells. These mechanisms of elimination of cells are non-specific. For example, tumor suppressors such as p53 use similar mechanisms for the eradication of damaged cells in order to maintain tissue homeostasis (Fridman and Lowe, 2003.; Halliwell, 2007.). Other researchers suggest that prevention of cancer by means of food rich in polyphenols may be due to their indirect antioxidant action. Frei and Higdon (2003.) were reviewing studies on the antioxidant activity of green tea and suggest that polyphenols can act indirectly as antioxidants, namely: a) inhibiting redox-sensitive transcription factors such as nuclear factor-kappaB and activator protein-1, b) inhibiting prooxidant enzymes such as inducible synthase nitric monooxides, lipoxygenase, cyclooxygenase, and xanthine oxidase, or c) inducing phase II enzymes and antioxidant enzymes such as GST and SOD. Such indirect antioxidant activity, which almost certainly could not be detected by conventional in vitro tests, could slow or stop cancer cell proliferation (Jia et al., 2002.; Hanif et al., 1997.). In recent decades, researchers and food manufacturers have shown great interest in the natural phenolic compounds. The main reason for this interest is their antioxidant activity, their representation in the human diet and their potential role in the prevention of various chronic diseases associated with oxidative stress. Consuming foods rich in natural antioxidants, as well as processed foods enriched with the same, ensure the desired supply of antioxidants and help prevent diseases in which oxidative stress is the key cause (Hardy, 2000.). Thus, natural sources of antioxidant polyphenols such as *E. purpuree* could be particularly significant and effective. Polyphenols are intentionally added to functional foods (Wyk and Wink, 2004; Buncova et al., 2008.; Sakač et al., 2005.; Huang et al., 2010.), and are often also found naturally in foods in quantities that have active effect on human health. Purple Echinacea herb extracts could serve as antioxidants that are added to prevent autooxidant food spoilage, but also as functional ingredients that can act antimutagenic and anticancerogenic and thus reduce the potential damage to the body.

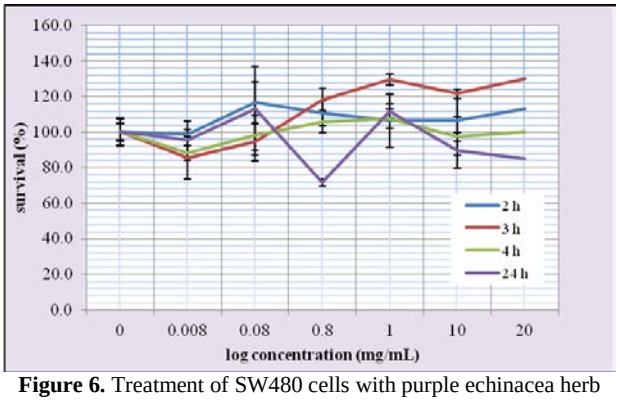


Figure 6. Treatment of SW480 cells with purple echinacea herb extract

CONCLUSIONS

The investigation of redox effect of Echinacea extract on cancer cells determined the prooxidant effect at lower concentrations and shorter duration of incubation. Higher concentrations and incubation of 4 hours or longer reduced the amount of ROS's in comparison to the control sample. Cells in which oxidative stress is fueled by hydrogen peroxide treatment showed a significant reduction in oxidative stress, which was particularly pronounced during longer incubation times. Based on the research of other authors, it can be assumed that with the time suppression of ROS`s had occured due to the induction of cellular antioxidant protection;

Treatment of cancer cells (SW480) with Echinacea extract for 2 and 3 hours showed a slight increase in the number of cells in the range of concentrations tested. After 24 hours of treatment a slight decrease in the number of surviving cells depending on a concentration was observed;

Due to a better understanding of the importance of polyphenols in the diet, it is necessary, beside bioavailability and mechanisms of action, to examine the possible synergism with other components present in food and the human body. Further investigations are required to identify a specific group of polyphenols or phenolic compounds that are most responsible for the positive effects on human health.

REFERENCES

Babich H, Borenfreund E, (1990). Applications of the Neutral Red cytotoxicity assay to in vitro toxicology. ATLA, 18:129-144.

Beatty ER, O'Reilly JD, England TG, McAnlis GT, (2000). Effect of dietary quercetin on oxidative DNA damage in healthy human subjects. *British Journal of Nutrition*, 84:919-925.

Buettner GR, (1985). Spin trapping of hydroxyl radical, in *CRC Handbook of Methods for Oxygen Radical Research*, Ed by Greenwald RA. CRC Press, Boca Raton, FL, 151-155.

Buřičova L, Reblova Z, (2008). Czech medicinal plants as possible sources of antioxidants. *Czech Journal of Food Sciences*, 26:132-138.

Colic M, Pavelic K, (2000). Molecular mechanisms of anticancer activity of natural dietetic products. *Journal of Molecular Medicine*, **78**:333-336.

Gugler RM, Leschik M, Dengler HJ, (1975). Disposition of quercetin in man after single oral and intravenous doses. *European Journal of Clinical Pharmacology*, **9**:229-234.

Finley JW, (2005). Proposed Criteria for Assessing the Efficacy of Cancer Reduction by Plant Foods Enriched in Carotenoids, Glucosinolates, Polyphenols and Selenocompounds. *Annals of Botany*, **95**:1075-1096.

Frei B, Higdon JV, (2003). Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. *Journal of Nutrition*, **133**:3275-3284.

Freshney RI, (2000). Quantitation -cell counting. In: Culture of animal cells. Wiley, J.,R.I. Freshney (eds). A John Wiley and sons INC., Publication NY; p, 309.

Fridman JS, Lowe SW, (2003). Control of apoptosis by p53. *Oncogene*, **22**:9030-9040.

Hail NJ, Cortes M, Drake EN, Spallholz EJ, (2008). Cancer chemoprevention: A radical perspective. *Free Radical Biology and Medicine*, **45**:97-110.

Halliwell B, Gutteridge JMC, (2007). Free Radical Biology and Medicine. 4th Ed., London, Oxford Univ Press.

Hardy G, (2000). Nutraceuticals and funcctional foods: introducing and meaning. *Nutrition*, **16**:688.

Hsu S, Yu FX, Huang Q, Lewis J, Singh B, Dickinson D, Borke J, Sharawy M, Wataha J, Yamamoto T, (2003). A mechanismbased in vitro anticancer drug screening approach for phenolic phytochemicals. *Assays in Drug Development Technology*, **1**:611-618.

Huang YC, Chang YH, Shao YY, (2006). Effects of genotype and treatment on the antioxidant activity of sweet potato in Taiwan. *Food Chemistry*, **98**:529-538.

Jia X, Han C, Chen J, (2002). Effects of tea on preneoplastic lesions and cell cycle regulators in rat liver. *Cancer Epidemiology Biomarkers and Prevention*, **11**:1663-1667.

Long LH, Hoi A, Halliwell B, (2010). Instability of, and generation of hydrogen peroxide by, phenolic compounds in cell culture media. *Archives of Biochemistry and Biophysics*, **501**:162-169.

Manach C, Williamson G, Morand C, Scalbert A, Rémésy C, (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *American Journal of Clinical Nutrition*, 81:230-242.

Mathew S, Abraham E, (2006). In vitro antioxidant activity and scavenging effects of *Cinnamomun verum* leaf extract assayed by different methodologies. *Food and Chemical Toxicology*, **44**:198-206.

Naczk M, Shahidi F, (2004). Extraction and analysis of phenolics in food. *The Journal* of *Chromatography A*, **1054**:95-111.

Niering P, Michels G, Watjen W, Ohler S, (2005). Protective and detrimental effects of kaempferol in rat H4IIE cells: implication of oxidative stress and apoptosis. *Toxicology and Applied Pharmacology*, **209**:114-122.

Teow CC, Truong V, McFeeters RF, Thompson RL, Pecota KV, Yencho GC, (2007). Antioxidant activities, phenolic and b-carotene contents of sweet potato genotypes with varyng flesh colours. *Food Chemistry*, **103**:829-838.

Zhou K, Yu L, (2006). Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado. *LWT - Food Science and Technology*, **39**:1155-1162.

Summary/Sažetak

U posljednjih nekoliko godina, postoji sve veći interes za prirodne i sigurnije antioksidanse. Do sada se malo zna o citotoksičnom i (anti) oksidativnom potencijalu echinacea (Echinacea purpurea) ekstrakata.

Metode: U cilju procijene antioksidativne aktivnosti ekstrakata, utvrđen je ukupan sadržaj fenola i ispiranje kapaciteta na DPPH radikalima. Sposobnost ekstrakata da izbace superoksid i hidroksilne radikale je testiran pomoću elektronske paramagnetske rezonancije (EPR) tehnike. Također, ekstrakti su prikazivani za citotoksičnost i antioksidativno/prooxidativni potencijal neutralnim crvenim i DCFDA testom, odnosno, koristeći liniju stanice ljudskog raka debelog crijeva SW480. Ćelije su izložene različitim koncentracijama ekstrakata (raspon: 0.008; 0.08; 0.8; 1, 10 i 20 mg/mL) i različito vrijeme tretmana (2, 3, 4 i 24 h).

Rezultati: Sadržaj ukupnih fenolnih jedinjenja ekstrakta E. purpurea je 10.57% GAE. U ispiranju aktivnosti radikala je utvrđeno da izlaže 50% od vrijednosti inhibicije (IC50 vrijednosti) u koncentraciji od 15.67 g/ml za ispitivane ekstrakt ehinaceae. Također, izračunata vrijednost 210 mg/ml za hidroksil i 76.7 mg/ml za superoksid anion radikale ukazuje na to da je ekstrakt Echinacea bogat antioksidans spojevima koji neutraliziraju radikale. U in vitro eksperimentima, ekstrakt ehinaceae pokazao prooxidativan učinak na nižim koncentracijama i kraći period inkubacije kada je korišten mobilni line SW480 kao test sistem. Najveća koncentracija bila je i najotrovnija što je posebno vidljivo nakon 24 sata tretmana.

Zaključci: Pokazalo se da Echinacea ekstrakt posjeduju jak antioksidans potencijal.

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2015

44

Print ISSN: 0367-4444

Online ISSN: 2232-7266

UDC: _____

Original Scientific Article

53-58

Mycelial growth rate and yield of oyster mushroom - *Pleurotus ostreatus* fruitful part (Jacquin: Fr.) Kumm at different temperatures

Turković, S.

Primary school "Edhem Mulabdić" Konak 1. Sarajevo, Bosnia and Herzegovina

Article info
Received: 21/4/2015
Accepted: 9/6/2015

Keywords:
mycelium,
oyster mushroom,
temperature,
nutrient medium,
yield,
substrate

***Corresponding author:**
E-mail: samir_t80@hotmail.com
Phone: 00-387-61-538386

Abstract: In this paper are presented possibilities for production of oyster mushroom, its mycelium and compost, as well as how different temperatures affect the mycelial growth and yield of these mushrooms. Mycelium was cultivated on the PDA media, which was later transferred onto the sterilized wheat grains and at the end in the compost made from beech sawdust and hay. The ratio of sawdust and hay was 1:1. Compost can be prepared from 50% wheat straw + 50% soybean straw, 50% wheat straw + 50% corn stems, 50% wheat straw and 50% sunflower stems (Bugarski et al, 2005).

The mycelium which was growing at an average temperature of 20 °C after 15 days was ready for the next grafting, while the mycelium, which was kept at 4 °C for the same period, had very slow growth rate. Mycelium was then inoculated onto sterile grain of wheat, which at a temperature of 20 °C grown very quickly. Two seedlings on compost were made, summer and autumn. The average air temperature during the summer seeding was 19.96 °C and in autumn 13.85 °C. It was found that the yield from the summer seeding was 17.76%, while the yield of autumn seeding was 7.2% relative to the weight of the wet substrate. Low temperatures, around 4ºC have inhibitory influence on the mycelium growth and in such conditions mycelium can be stored up to one year. The average temperature of 19.96 °C is ideal for the growth of both mycelium and mushrooms, as well as the expected yield.

INTRODUCTION

Today in controlled conditions about 30 species of fungi can be successfully cultivated. The basic preconditions for the artificial production of mushrooms are: the choice of substrate for the cultivation, selection of the mushroom type and control of growth conditions. Majority of world mushroom production is dedicated to the noble mushrooms. In second place, in terms of produced mushrooms quantity, are oyster mushrooms followed by a shiitake (Novak, 1997). To be able to cultivate specific sort of mushroom it is necessary to know its life cycle. A relatively simple method of production and high nutritional value are sufficient reasons for this type of research. During cultivation when the parameters, such as light, temperature and humidity, are controlled yields are much higher. Given the above facts as well as economic importance of the complete production of this mushroom, the following basic goals of this study are set: to master the technique of growing mycelium in vitro, to produce a substrate suitable to inoculate already produced mycelium, to monitor the growth rate of mycelium in laboratory conditions, to follow temperature during growth, record the changes and get healthy fertile mushroom body. For the production of oyster mushroom the substrate is prepared from herbal residues like: wheat, soya, rice, straw, bean, pea, cotton stems, and other waste parts of the industry such as: sugar cane, sunflower husks and stems, etc. (Bugarski et al, 2002).

Oyster mushroom, which is produced on various types of residues from the cellulose, has a high proportion of protein, vitamin C, D and B complex. Vitamin A is absent (Jonathan et al, 2012).
In nature, the oyster is widespread in many different trees, often as a parasite. It usually grows on beech, warm, walnut, willow, black locust and oak trees and rarely on conifers and maple (Focht, 1990). Oyster mushroom grows in cups, in the autumn until the first frosts, and even in winter, if it is mild; on stumps and trunks of harvested trees (Pace, 1981).

MATERIAL AND METHODS

Parent mycelium was isolated from individual oyster mushroom from the surrounding area of Konjic cityand used in this study. The mycelium was transported in sterile test tube on a PDA (potato dextrose agar) nutritive surface (Figure 1). All the containers and instruments were sterilized in an autoclave. Sterilization was performed at a temperature of 135 ºC and a pressure of 2 Atm for 60 minutes from the moment of achieving the above mentioned parameters. Nutrient media were also sterilized in an autoclave. After sterilization substrate was placed into Petri dishes and test tubes, while sterile nutrient medium was inoculated with obtained mycelium in sterile chamber (Vukojevic, 2000).

Figure 1. A parent mycelium

When an inoculum mycelium grown through the nutrient medium it is ready for further grafting, or to be shift to another carrier media. In this study, a sterile wheat grain was used as a second carrier. Wheat grains were prepared to plant mycelium on them in the manner that they were first dipped in hot water and left for 24 hours, during which time the wheat swell. This stage is important because the water from the swollen wheat is used by mycelium for its normal growth and development. After 24 hours, the wheat was thoroughly washed and sterilized in an autoclave. Sterilization was performed in duration of 30 minutes at a temperature of 114 ºC and a pressure of 1 atm. In this study, as containers for sterilization of mycelia carriers were used glass jars with volume of 0.3L and 0.2L with polypropylene lid and the jar of larger volume without polypropylene lids. The lids on jars during sterilization were not completely closed due to the equalization of pressure inside and outside of the jars during the sterilization process. Upon completion of the sterilization lids were quickly and completely closed and allowed to cool. When the temperature of the grain in the pots decreased below 30 ºC, followed the phase of mycelium transplantation from the Petri dish with substrate on wheat grain in sterile conditions.
In this study, for the preparation of compost was used hay and beech sawdust in the 1:1 ratio. Compost was cooked for two hours with the lid closed in order that inside a pot, in which it was cooked, increased pressure also increase the effect of pests' destruction. After cooking the pot remained closed to gradually cools and thus prolonging thermal treatment. When the substrate was cooled below 30 ºC the seeding started.
Seeding of compost with mycelium is the most sensitive stage in the production, because of frequent infections by competitive species of mushrooms and some types of bacteria. After the technical preparation of compost, it was seed with mycelium and packed in nylon bags with volume of 10L. Seeding is done by mixing compost with mycelium on nylon. The substrate was seeded with mycelium in ratio 5-7% by weight of the wet substrate (Smith, 1997). After seeding and packaging of compost, each bag weighed 15 kg.

RESULTS

After inoculation of the sterile substrate with a piece of mycelium its growth was followed at room temperature (20 °C) and in the refrigerator (4 °C). Average daily growth (ADG) of the mycelium at 20 °C was approximately 3 mm on all edges of the seeded surface. On the seventh day after inoculation mycelium covered surface of 18 mm, extending concentrically around the inoculum. At this rate of growth, after 15 days the mycelium was ready for the next grafting (Figure 2).
Mycelium best develop at a temperature of 25 °C and fully outgrowth substrate mycelium on the 15th day after inoculation. Growth is optimal at temperature of 25 °C, followed by growth rate at a temperature of 20 °C, while growth at 30 °C was the weakest (Bugarski et al, 1997).

In case of the mycelium which was left in the refrigerator immediately after seeding the first sign of growth was noticed seven days after inoculation in a form of off white thickening at the edge of the inoculums (Table 1).

Figure 2. The substrate covered with mycelium

As can be observed the low temperature (4 °C) has an inhibitory activity on the mycelium growth by slowing down physiological processes, which slow use of nutrient medium, so that mycelium in these conditions (4 °C) can survive for up to one year. Mycelial growth at the stage when it was placed on sterile grain was at the same rate as in the case with Petri dishes (at a temperature of 20 °C) and after 15 days the mycelium was ready for the next grafting.

Table 1. Growth of mycelium at a temperature of 4 °C and 20 °C.

Day	Temperature 4^0 C	Temperature 20^0 C
1.	---	---
2.	---	White thickening of the inoculum edge
3.	---	2 – 3 mm
4.	---	5 – 6 mm
5.	---	10 mm
6.	---	15 mm
7.	White thickening of the inoculum edge	18 mm

Having examined the influence of temperature on growth, two experimental seedlings were performed. In each experiment 10 bags were sown. First seeding was done in the summer and the other in the autumn. The average temperature during incubation and fructification in the two cycles varied considerably as shown in Figure 3.

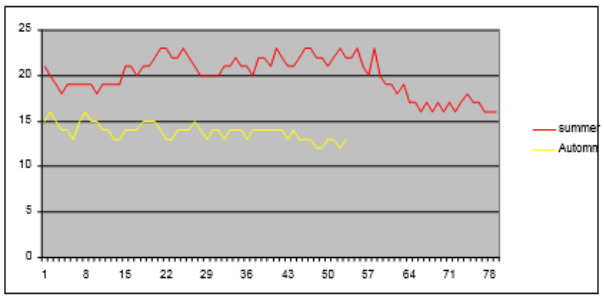


Figure 3. Temperature variations according to seasons

Temperature of the room for outgrowth (incubation) should be between 18 ^{0}C and 20 ^{0}C (Novak, 1997). The temperature of the room in which the incubation took place for the first experiment, and which lasted for 79 days, ranged from 16-23 ^{0}C (with an average temperature of 19.96 ^{0}C), which does not represent a radical deviation from the optimum temperature as reported by Novak (1997). Reported incubation duration is 15-25 days (Novak, 1997). In this experiment, incubation lasted 29 days, which also does not represent significant deviation from the above mentioned literature references.

After incubation began fruiting phase (fructification) or its first cycle (Figure 4). The first cycle usually provide about 70% of the total yield of mushrooms, in the second cycle can be expected 20 to 25% and in the third 10 to 15% of the total yield of mushrooms. The yield is calculated based on the weight of the wet substrate, ranging from 15 to 30% from weight of the wet substrate. Weight of moist substrate in this experiment was 15 kg for each of the 10kg bags. Expected yields on the above literature data, cited by Novak (1997), would amount to 2.25 to 4.50 kg per bag (15-30 % of 15 kg, which was the weight of the wet substrate).

Figure 4. Start of fructification

Weight of the mushrooms in the first cycle of the first experimental seeding can be seen in Table No. 2 for each bag separately while the average yield of the first cycle was 1.77 kg per bag.

In the second cycle the mushrooms began to produce yield after 49 days from the day of compost inoculation. The yield of mushrooms harvested in this cycle is shown in Table 2. In this cycle of harvesting mushrooms average yield per bag was 0.58 kg. The harvest was short, only two to three days, so that the third cycle of fruiting started 55 days after inoculation. The third cycle of fruiting was mixed in the sense that all the bags did not yield simultaneously. This cycle is completed 69 days after inoculation, when the harvest was performed on the last bag. The yield of the third fructification cycle was slightly smaller than the yield from others, as shown in Table 2. The average number of yield in the third harvest was 0.325 kg per bag.

Adding up all the yields from all three cycles of fruiting we obtained information that the total yield per bag was 2.665 kg, which is 17.76% of the moist compost weight. The values obtained agree with the data cited by Novak (1997), which was expected because the parameters (temperature, mycelium-compost ratio) did not significantly differ from the optimal value.

The incubation at the room temperature in the second experimental seeding, which was done in the autumn, ranged from 12 to 16 °C. The average incubation room temperature was 13.85 °C, which is significantly lower than the optimum temperature (18-20 °C). The lower temperature has the effect of extending the incubation time and reducing the strength and vitality of the mycelium (Novak, 1997). Incubation in the second experimental seeding lasted 44 days, when first primordia appeared. Only 53 days after inoculation the mushrooms were ready for harvesting. Due to the low temperatures, fruiting after the first cycle was no longer existent. The yield was much lower than the yield of the first experimental seeding (Table 3) and amounted to only 7.2% relative to the weight of the wet substrate.

The average yield per bag from another experimental seeding was 1.080 kg, which represents 40.52% of the yield obtained in the first experimental seeding.

Table 2. The weight of yield of summer seeding per cycle (kg)

bag	1	2	3	4	5	6	7	8	9	10	∑	X
1. cycle	1.75	1.45	1.90	1.65	1.90	1.50	1.85	1.70	2.20	1.75	17.65	1.77
2. cycle	0.55	0.50	0.70	0.45	0.65	0.40	0.65	0.50	0.70	0.65	5.75	0.58
3. cycle	0.35	0.30	0.40	0.30	0.35	0.25	0.45	0.30	0.35	0.20	3.25	0.33
∑	2.65	2.25	3.00	2.40	2.90	2.15	2.95	2.50	3.25	2.60	26.65	2.67

Table 3. The weight yield of autumn seeding (kg)

Bag	1	2	3	4	5	6	7	8	9	10	∑	X
Yield	1.50	1.20	0.90	0.80	1.30	1.20	1.10	1.00	0.60	1.20	10.8	1.08

CONCLUSION

When examining the effect of different temperatures on the mycelial growth we found that: low temperature (4 °C) slows down the growth of the mycelium; at a temperature of 20 °C, the mycelium grows relatively quickly and the mycelium grow was at the same rate on a sterile wheat grain as well as the culture medium, at the same temperature (20 °C), with incubation at a temperature which varied between 16-23 °C and with an average of 19.96 °C. The incubation was completed after 29 days, at a temperature which varied between 12 and 16 °C (the mean value 13.85 °C) after incubation of 44 days. Yield of the first (summer) experimental seeding was 17.76 % of the wet substrate mass. The yield of the second (autumn) experimental seeding was 7.2 % based on the weight of the wet substrate. In general, we can conclude that in the production of oyster mushrooms it is required to ensure during all phases of the growth the temperature of about 20 °C (without major deviations) in order to obtain optimum yield.

REFERENCES

Bugarski, D., Gvozdenović, Đ & Jovićević, D. (2002) Upotreba ostataka biljne proizvodnje kao i neiskorištenih objekata u proizvodnji gljiva. *Zbornik radova Instituta za ratarstvo i povrtarstvo* no. 36, pp. 75-84

Bugarski, D., Gvozdenović, Đ., Červenski, J. & Takač, A. (2005) Supstrat kao faktor prinosa u proizvodnji bukovače (Pleurotus ostreatus). *Zbornik radova Instituta za ratarstvo i povrtarstvo*, no. 41, pp. 119-127

Bugarski,D. Gvozdenović,Đ., Vasić Mirjana,Jovićević,D. (1997). Međuzavisnost činioca koji utiču na razvoj micelija gljive bukovače (Pleurotus ostreatus). *Selekcija i seminarstvo*, no 3-4, pp. 147-151

Focht, I. (1990).Ključ za gljive. *ITP Naprijed*, Zagreb

Novak, B. (1997). Uzgoj jestivih i ljekovitih gljiva. *Hvatsko agronomsko društvo*, Zagreb

Pace, G. (1981).Atlas gljiva . *Prosvjeta*, Zagreb.

Vukojević, J. (2000).Praktikum iz mikologije i lihenologije , *NNK Internacional*, Beograd

Jonathan S. G., Okon C.B., Oyelakin, A.O., Oluranti O.O.(2012).Nutritional values of oyster mushroom (Pleurotus ostreatus) (Jacq. Fr.) Kumm. cultivated on different agricultural wastes. *Nature & Science*; 2012, Vol. 10 Issue 9, p186

Summary/Sažetak

Mogućnosti proizvodnje gljive bukovače, njenog micelija i komposta, te kako različite temperature utiču na rast micelija i prinos gljive, predstavljene su u ovom radu. Na PDA podlogama razmnožen je micelij, koji se kasnije prebacivao na sterilisana zrna pšenice, te na kraju u kompost od bukove piljevine i sijena. Omjer piljevine i sjena bio je 1:1. Kompost se može pripremiti i od pšenične slame 50% i sojine slame 50%, pšenične slame 50% i stabljike kukuruza 50%, pšenične slame 50% i stabljike suncokreta 50% (Bugarski i ostali, 2005).

Micelij je brzo rastao na prosječnoj temperaturu od 20 ^{o}C i nakon 15 dana je bio spreman za naredno presađivanje, dok je micelij koji je držan na 4 ^{o}C za to vrijeme rastao zanemarivo malo. Micelijem je dalje inokulisano sterino pšenično zrno, gdje je na temperaturi od 20 ^{o}C rastao veoma brzo. Na kompostu su bila dva zasijavanja, ljetno i jesensko. Prosječna temperatura zraka u toku ljetnog zasijavanja je iznosila 19,96 ^{o}C, a a kod jesenskog 13,85 ^{o}C. Ustanovljeno je da prinos u ljetnom zasijavanju je bio 17,76%, dok je prinos jesenskog zasijavanja bio 7,2% u odnosu na težinu vlažnog supstrata. Niske temperature, oko 4 ^{o}C inhibitorno utiču na rast micelija, te se na takav način micelij može čuvati i do jednu godinu. Prosječna temperatura, 19,96 ^{o}C je idealna za rast i razvoj micelija i gljive, te daje očekivane prinose plodonosnih tijela.

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2015

44

Print ISSN: 0367-4444

Online ISSN: 2232-7266

UDC: _____

Original Scientific Article

59-64

Changes in lecithin concentrations in human blood with aging

Ajanović, A.[a], Sofić, E.[a,b], Tahirović, I.[a*], Šapčanin, A.[a], Uzunović, A.[a], Krehić, J.[c], Gojak, R.[c], Dizdar, M.[a]

[a]*University of Sarajevo, Faculty of Sciences, Zmaja od Bosne 33-35, Sarajevo, Bosnia and Herzegovina*

[b]*University of Sarajevo, Faculty of Pharmacy, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina*

[c]*Clinical Center University of Sarajevo, Bolnička 25, Sarajevo, Bosnia and Herzegovina*

Article info
Received: 29/4/2015
Accepted: 29/6/2015

Keywords:
phospholipids
lecithin
human plasma
human whole blood
gender
age

***Corresponding author:**
E-mail: esofic@pmf.unsa.ba
ismet_tahirovic@yahoo.com
Phone: 00-387-33-279897
Phone: 00-387-33-279905
Fax: 00-387-33-649359

Abstract: Lecithin is a phospholipid and a major structural component of the cell membrane. The aim of this study was to determine the lecithin concentrations (LC) in the human blood depending on age. Lecithin was measured in the human plasma (807) and whole blood (787) of men and women aged 0.6 to 90 years, by using a commercially available enzymatic kit, which is based on the spectrophotometric determination. All samples were divided into four aging groups: children (0-12 years), adolescents (13-20 y.), work-active group (21-60 y.), and older group (>60 y.). The plasma LC of all males (397) were very similar to those of females (410) (mean 3.01±0.91 mmol/l, and 2.99±1.02 mmol/l, respectively). However, within both gender, the plasma LC increased with aging. The LC in whole blood increased with aging in males, but in the work-active group, and in older group of women, the whole blood lecithin decreased without statistical significance. Also, the LC in whole blood of older women were statistically lower than the LC in older men ($p^{***}<0.001$, Mann-Whitney test), while the LC in whole blood of other groups of both gender were mainly uniform. The lecithin ratio in plasma and whole blood was ≈1:2 in all aging groups.

INTRODUCTION

Phospholipids (PL) are the major structural components of the cell membranes. Numerous data indicate that in cells throughout life, *in vivo*, the damage accumulates, leading to a gradual loss of differentiated function and the degree of growth (Boyer, 2006). These changes lead to breakdown of normal homeostatic mechanisms. Gradual reduction of cellular functions and reducing the capacity of the cell growth with aging, are based on the discoordination of interactive pathways in the cells themselves, as well as between cells and tissues (Bourne, 2012). PL are unevenly distributed in the blood. In plasma, they make up about 35-40% of total lipids, 60-65% in erythrocytes, leukocytes 50%, and platelets up to 70% of total lipids. In the plasma, approximately 50% of total PL are in the form of lecithin. Plasma containing normal 2.3-4.0 mmol/l PL (Straus, 1992). With aging of the organism, red blood cells (RBC) also change their shape, deformability, fragility and fluidity of their membranes (Marin et al., 1990). Aging of the RBC can easily be understood in two ways. The first, is to have a life cycle of the individual RBC, which lasts about three months, and the second: the change in erythropoiesis of the older RBC. In the first case, the reduced fluidity of RBC membranes of elderly in particular relating to the account of reduction in the ratio of lipid/protein, reduced levels of ATP and changed the shape of RBC (Shiga et al., 1979).

In the second case, reduced fluidity is associated with a reduced ratio of phosphatidylserine/sphingomyelin, which increases rigidity. Also, the impact of the changes in membrane lipid peroxidation (LP) with aging has great importance and contributes to higher rigidity of the membrane (Marin et al., 1990). Increased LP increases the risk of atherosclerosis and other inflammatory diseases (Stefan et al., 2007; Halliwell and Gutteridge, 1984).

Increased amounts of transmembrane proteins has resulted in the creation of protein-lipid domains, which reduce membrane dynamics (Subczynski and Wisniewska, 2000). The aim of this study was to determine the LC in both human blood plasma and in whole blood in different aging groups of people.

MATERIAL AND METHODS

A total of 807 blood plasma samples and 787 whole blood samples of both gender aged from 0.6 to 90 years were analysed by using an enzymatic-spectrophotometric method, (Takayama et al, 1977) with a commercial kit (Chronolab Systems, Spain) on UV/VIS spectrophotometer (Perkin Elmer Lambda 25).

The enzymatic method uses a combination of two purified enzymes, phospholipase D and cholinoxidase that through a sequence of reactions produce H_2O_2. The resulting H_2O_2 reacts with the mixture of 4-aminoantipyrine and dichlorophenol (reaction is catalysed by peroxidase, POD) to give quinone imine, which absorbs at 505 nm. The absorption of quinone imine is proportional to the concentration of phospholipids:

$$\text{choline phospolipids} + H_2O \xrightarrow{phospholipaseD} \text{choline} + \text{phosphatidic acid ester}$$

$$\text{choline} + 2O_2 + H_2O \xrightarrow{cholinoxidase} \text{betaine} + 2H_2O$$

$$2H_2O_2 + \text{4-AP} + \text{dichlorophenol} \xrightarrow{POD} \text{quinone imine} + 4H_2O$$

For the determination of lecithin in whole blood, all specimens were diluted with double volume of 0.9% NaCl solution. Statistical analysis was performed using Descriptive Statistics (Kruskal-Wallis test and Mann-Whitney test), using the statistical program SPSS 19.0 for Windows.

Ethics

All specimens sampled in the Laboratory for Clinical Chemistry and Biochemistry of the Clinical Centre University of Sarajevo and approved by the Ethics Committee of the Clinical Centre University of Sarajevo (Nr. 0305-33560 on December 18, 2006.).

RESULTS AND DISCUSSION

The results of the concentration of lecithin in the blood plasma and in whole blood in human subjects of different aging groups are shown in Table 1.

Table 1. *The lecithin concentrations in the plasma and in whole blood in different aging groups of people*

Age (y.)	n	Plasma lecithin (mean±S.D.) (mmol/l)	n	Whole blood lecithin (mean±S.D.) (mmol/l)
≤12	92	1.57±0.61	94	4.39±0.74
13-20	95	2.71±0.65***	95	5.70±0.70***
21-60	351	3.22±0,87***a	346	5.76±1.09***
>61	269	3.30±0.82***	252	5.73±1.11***
	Total (n=807)	3.00±0.96	Total (n=787)	5.58±1.11

*** Significantly higher concentrations of lecithin in comparison to the youngest group of subjects (p<0.001, Mann-Whitney test)
[a] Significantly higher concentrations of lecithin in comparison to the adolescent group of subjects (p<0.01, Mann-Whitney test)

Results of this study showed that the concentration of lecithin in the whole blood and in plasma of total subjects (without taking into account the gender) statistically significantly increases after 12 years of age (p***<0.001).
In aging group 21-60 y. (total, without taking into account the gender), the lecithin concentration in the plasma increases (p***<0.001), and after age 60 (older group, >60 y.) the concentration of lecithin was slightly increased in plasma, and slightly decreased in whole blood in comparison to the work-active group. The ratio of lecithin in plasma and whole blood of both gender was ≈ 1:2 and that relationship did not change during aging.
The results of the concentrations of lecithin in the blood plasma of males and females of different aging groups are shown in Table 2.

Table 2. The lecithin concentrations in the plasma of different aging groups for both gender

Age (y.)	n	Plasma lecithin (mean±S.D.) (mmol/l) Men	n	Plasma lecithin (mean±S.D.) (mmol/l) Women
≤12	46	1.62±0.64	46	1.52±0.58
13-20	47	2.61±0.69***	48	2.81±0.60***
21-60	169	3.25±0.78***	182	3.19±0.94***
>60	135	3.31±0.71***b	134	3.29±0.92***
	Total (n=397)	3.01±0.91	Total (n=410)	2.99±1.02

*** Significantly higher concentrations of plasma lecithin in comparison to the youngest group of subjects (p<0.001, Mann-Whitney test)
[b] Significantly higher concentrations of plasma lecithin in comparison to the adolescent group of subjects (p<0.01, Mann-Whitney test)

The concentrations of lecithin in the blood plasma of total male subjects (n=397, mean 3.01±0.91 mmol/l) were not significantly different from the concentrations of lecithin of total female subjects (n=410, mean 2.99±1.02 mmol/l).

The concentrations of plasma lecithin in subjects of both gender were statistically significantly increased after 12 years (p***<0.001, Mann-Whitney test). Also, the concentrations of plasma lecithin in the work-active and older group of subjects of both gender were statistically significantly increased in comparison to the adolescent group (p***<0.001, and p**<0.01 for older group of women to adolescent group; Mann-Whitney test).
There were no statistically significant differences in the plasma lecithin concentrations of male and female subjects in the same aging groups.

The results of the concentrations of lecithin in the whole blood of males and females of different aging groups are shown in Table 3.

Table 3. The lecithin concentrations in the whole blood of both gender of different aging groups

Age (y.)	**n**	**Whole blood lecithin (mean±S.D.) (mmol/l) Men**	**n**	**Whole blood lecithin (mean±S.D.) (mmol/l) Women**
≤12	49	4.39±0.73	45	4.38±0.76
13-20	47	5.58±0.70***	48	5.82±0.69***
21-60	171	5.83±1.18***	175	5.69±1.01***
>60	125	6.03±0.96*** c	127	5.44±1.18**d
	Total (n=392)	5.67±1.12**e	Total (n=395)	5.48±1.08

*** Significantly higher concentrations of lecithin in whole blood in comparison to the youngest group of subjects (p<0.001, Mann-Whitney test)
** Significantly lower concentrations of lecithin in comparison to those of the youngest group of female subjects (p<0.01, Mann-Whitney test)
c Significantly increased in comparison to the adolescent group (p<0.01, Mann-Whitney test)
d Significantly lower concentrations of lecithin in whole blood in comparison to the same aging group of men (p<0.01, Mann-Whitney test)
**e Significantly higher than LC of total female subjects (p<0.01, Mann-Whitney test).

The concentration of lecithin in whole blood of total male subjects (n=392, mean 5.67±1.12 mmol/l) were statistically higher in comparison to those of total female subjects (n=395, mean 5.48±1.08 mmol/l) (p**<0.01, Mann-Whitney test).
The whole blood LC in all groups of subjects of both gender were statistically significantly higher in comparison to the youngest group of subjects (p*** <0.001). Also, the whole blood LC in the older group of male subjects were statistically significantly increased in comparison to the adolescent group (p**<0.01, Mann-Whitney test). Decreasing of the whole blood LC in the older group of female subjects was marginally significant in comparison to the adolescent group (p=0.058, Mann-Whitney test).
Also, the whole blood lecithin concentrations of older female subjects (>60 y.) were statistically significantly lower than those in the same aging group of males (p***<0.001, Mann-Whitney test), while the whole blood lecithin concentrations in other aging groups of both gender were generally uniform.
Satisfactory results were obtained, which correlate with other previously used reference methods. By separating the plasma lipids using by agarose gel electrophoresis and measured by reference methods, it was found that in infants at birth, the concentration of PL in the blood plasma is very low (1.36±0.18 mmol/l) (Gurantz et al., 1981). After about one month of life, plasma phospholipid concentrations reach values twice as high as the initial value (Wissenschaftliche Tabellen Geigy, 1986).
The earlier studies have shown that during the first year of life, plasma PL concentrations continuously increasing, it would have reached puberty, values that are common in young adults (Wijnberger et al., 2003). For boys younger than 15, the plasma PL concentrations were lower compared to girls of the same age. From age 16-50 the concentrations of plasma PL were approximately the same in both gender with a tendency of gradual increase during aging. In this age period, the PL concentrations ranged from 2.36 to 2.98 mmol/l for both gender. After age 50 for both gender, the concentration of plasma PL ranged from 3.28-3.30 mmol/l (Wissenschaftliche Tabellen Geigy, 1986). According to data from the American Heart Association (Heistad, 2006), 4-5 people who died of heart diseases are over 65 years old. Heart diseases commonly occur more often in younger men, than in women. The risk for them is increasing especially after 45 years of age. In women, the risk for these diseases increases sharply after age 55 (Knight, 2000). The reason for this protective effect (in comparison to 45 y. for men) is estrogen, but when women reach menopause when estrogen secretion is reduced, they are much more prone to cardiovascular diseases (CVD). Estrogen changes the concentration of lipids in the blood, affects the coagulation and fibrinolytic systems, antioxidant systems and the production of other vasoactive molecules such as prostaglandins, prostacyclins, tromboxanes etc. As for arterial hypertension, which is the most common cause of CVD, investigations suggest that the cause can easily be variability of erythrocyte membranes. Studies have shown that people with hypertension have less fluid membranes (Tsuda et al., 2003). After menopause, the use of hormon replacement therapy (HRT) may have a protective effect against CVD in women (Canadian Medical Association Journal, 2004). Estrogen stimulates the opening of calcium activated K-channels, K^+ out of cells, the membrane becomes hyperpolarized, leading to vasodilatation (Grant and Beastall, 1983).

CONCLUSIONS

The concentration of lecithin in the whole blood and plasma of total subjects (regardless of gender) statistically significantly increases after 12 years of age (p***<0.001). After 20 years, the concentration of lecithin in whole blood and plasma of subjects regardless of gender, is growing, but without statistical significance between age groups. The plasma lecithin concentrations of total male subjects were not significantly different from those of total female subjects (3.01±0.91 mmol/l, and 2.99±1.02 mmol/l respectively). There were no statistically significant differences in the plasma lecithin concentrations of the same aging groups of male and female subjects. The concentration of lecithin in whole blood of total male subjects was statistically higher in comparison to the lecithin concentration of total female subjects (5.67±1.12 mmol/l, and 5.48±1.08 respectively; p**<0.01, Mann-Whitney test). The concentrations of lecithin in the whole blood of older female subjects (>60 y.) were statistically significantly lower than those in the same aging group of males (p***<0.001, Mann-Whitney test). The ratio of lecithin in plasma and whole blood in subjects of both gender was ≈1:2, and it was found that this ratio did not alter during aging. The changes of lecithin concentrations in the blood during the aging processes can be partly explained by cell membrane dysfunction and provide insight into the metabolic and biochemical disturbances in the process of aging. Because that, we can reasonably say that the changes of lecithin concentrations in the plasma and whole blood during of aging are very important risk factor of various cardiovascular, neurodegenerative, diabetic and malignant diseases, general diseases of aging etc. The changes could be used as a diagnostic biochemical indicator of the above-mentioned pathochemic states, as well.

ACKNOWLEDGEMENT

The research was supported by Ministry for Education, Science and Youth of the Sarajevo Canton (Contract about funding and realisation of scientific-research work, Nr. 11-14-21627.1/07 on December 27, 2007.).

REFERENCES

Bourne, G. (2012). *Cytology and cell physiology*. Elsevier.

Boyer, R. F. (2006). *Concepts in biochemistry*. (3rd Ed), Wiley & Sons.

Canadian Task Force on Preventive Health Care (2004). Postmenopausal hormone replacement therapy for primary prevention of cardiovascular and cerebrovascular disease: Recommendation statement from the Canadian Task Force on Preventive Health Care. *Canadian Medical Association Journal*, *170(9)*, 1388-1389.

Grant, J. K., Beastall, G. H. (1983). *Clinical Biochemistry of Steroid Hormones: Methods and Applications*. Elsevier Science & Technology.

Gurantz, D., Laker, M. F., Hofmann, A. F. (1981). Enzymatic measurement of choline-containing phospholipids in bile. *Journal of lipid research*, *22(2)*, 373-376.

Halliwell, B., Gutteridge, J. C. (1984). Lipid peroxidation, oxygen radicals, cell damage, and antioxidant therapy. *The Lancet*, *323(8391)*, 1396-1397.

Heistad, D. D. (2006). Oxidative Stress and Vascular Disease 2005 Duff Lecture. *Arteriosclerosis, thrombosis, and vascular biology*, *26(4)*, 689-695.

Knight, J. A. (2000). The biochemistry of aging. *Advances in clinical chemistry*, *2001*(35), 1-62.

Marin, M. S., Fernandez, A., Sanchez-Yagüe, J., Cabezas, J. A., Llanillo, M. (1990). Changes in the phospholipid and fatty acid composition in normal erythrocytes from sheep of different ages. Aminophospholipid organization in the membrane bilayer. *Biochimie*, *72(10)*, 745-750.

Shiga, T., Maeda, N., Suda, T., Kon, K., Sekiya, M. (1979). The decreased membrane fluidity of *in vivo* aged, human erythrocytes. A spin label study. *Biochimica et Biophysica Acta*, *553(1)*, 84-95.

Stefan, L., Tepsic, T., Zavidic, T., Urukalo, M., Tota, D., Domitrovic, R. (2007) Lipid peroxidation-causes and consequences. *Medicina*, *43*, 84-93.

Subczynski, W. K., Wisniewska, A. (2000). Physical properties of lipid bilayer membranes: relevance to membrane biological functions. *Acta Biochim Pol*, *47(3)*, 613-625.

Takayama, M., Itoh, S., Nagasaki, T., Tanimizu, I. (1977). A new enzymatic method for determination of serum choline-containing phospholipids. *Clinica Chimica Acta*, *79(1)*, 93-98.

Tsuda, K., Kinoshita-Shimamoto, Y., Mabuchi, Y., Nishio, I. (2003). Hormone replacement therapy improves membrane fluidity of erythrocytes in postmenopausal women: an electron paramagnetic resonance investigation. *American journal of hypertension*, *16(6)*, 502-507.

Wijnberger, L. D. E., de Kleine, M., Voorbij, H. A. M., Arabin, B., van de Leur, J. J. C. M., Bruinse, H. W., Visser, G. H., Bossuyt, P. M., Mol, B. W. (2003). The effect of clinical characteristics on the lecithin/sphingomyelin ratio and lamellar body count: a cross-sectional study. *Journal of Maternal-Fetal and Neonatal Medicine*, *14(6)*, 373-382.

Wissenschaftliche Tabellen Geigy (1986) Achte, revidierte und erweiterte Auflage. Blut-Lipide. *Ciba Geigy AG*, 114-120.

Summary/Sažetak

Lecitin je fosfolipid i glavna strukturna jedinica ćelijske membrane. Cilj ovog istraživanja je bio odrediti koncentracije lecitina (KL) u ljudskoj krvi u ovisnosti od starosne dobi. KL je određivana u krvnoj plazmi (807) i punoj krvi (787) muškaraca i žena starosne dobi 0,6-90 godina, spektrofotometrijskom metodom uz komercijalno dostupni enzimatski reakcioni kit. Svi analizirani uzorci podijeljeni su u sljedeće starosne kategorije: djeca (0-12 godina), adolescenti (13-20 godina), radno-aktivnu grupu (21-60 godina) i grupu starijih osoba (>60 godina). KL u krvnoj plazmi muškaraca (397) i žena (410) je vrlo slična (3,01±0,91 mmol/l i 2,99±1,02 mmol/l respektivno). Međutim, kod oba spola, KL krvne plazme je rasla sa povećanjem starosne dobi. KL u punoj krvi je rasla sa povećanjem starosne dobi kod muškaraca, dok u radno-aktivnoj grupi i grupi starijih žena KL opada bez statističke značajnosti. Pored toga, KL u punoj krvi grupe starijih žena je statistički značajno niža u odnosu na KL u punoj krvi grupe starijih muškaraca ($p^{***}<0.001$, Mann-Whitney test), dok je u ostalim kategorijama KL u punoj krvi kod oba spola bila uglavnom ujednačena. Odnos KL u krvnoj plazmi i punoj krvi u svim starosnim grupama bila je ≈1:2.

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2015

44

65-70

Print ISSN: 0367-4444
Online ISSN: 2232-7266

UDC: _____

Original Scientific Article

Students with disabilities and chemistry education: Possibilities and difficulties

Zejnilagić-Hajrić, M., Delić, E., Nuić, I.

University of Sarajevo, Faculty of Science, Department of Chemistry, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina

Article info
Received: 10/3/2015
Accepted: 29/6/2015

Keywords:
Students with disabilities, students with hearing impairments, students with visual impairments, university study of chemistry, general chemistry

***Corresponding author:**
E-mail: mzejnilagic@pmf.unsa.ba
Phone: 00-387-33-279864
Fax: 00-387-33-649359

Abstract: Education of students with disabilities in B&H is regulated by law for primary and secondary education by responsible institutions (ministries). It can be implemented in regular schools with or without adopted curriculum, and in special centers for their education. This paper presents results of study conducted in two centers: for secondary school students with visual (CSSDO) and hearing (CSGR) impairments. The aim of the study was to explore their knowledge and interest in studying chemistry at university level. Results showed: (1) there is no significant difference in students' achievements on knowledge test in general chemistry (GC) in CSSDO and CSGR, (2) considering their achievements in GC, they have a chance to enroll to university majoring in chemistry based on earlier entrance exams, (3) majority of students would like to enroll to university after secondary school, (4) but only one student would consider studying chemistry. These results show significant obstacles for students with disabilities to enroll to university, especially when studying science, but also the lack of proper education for teaching staff both at university and in secondary school when it comes to education of students with disabilities.

INTRODUCTION

Education for children with disabilities until 1960s have been carried out in special institutions in segregated educational system, aimed to "fix" the child and to prepare her/him for the community. Nowadays, a large number of children with disabilities are educated in regular classrooms around the world, with the help of teachers and teaching assistants, with main goal being to help students with disabilities to improve their knowledge and quality of their education by individualization of teaching and learning process.

It is essential to properly define and standardize the terminology related to persons with disabilities.

There are a number of terms that are more or less usual in everyday language. It should be pointed out that different terms are considered to be adequate and proper in different countries. Disability is defined as a "physical or mental impairment that substantially limits an individual in performing one or more 'major life activities'. These include everyday activities such as caring for oneself, performing manual tasks, walking, seeing, hearing, speaking, breathing, learning, and working." (Miner et al., 2001). This term includes a variety of congenital and acquired defects different types and levels, such as hearing, vision or speech defects communication disability, intellectual disability, different brain lesions that manifest as difficulty of movement, damage to muscles and nerves (cerebral paralysis), or in communication and social skills overcoming disability (autism) (DUGA, 2013).

Children and young people with special educational or rehabilitation needs are those who, in order to achieve optimal development capabilities and other social and personal positive characteristics of personality, need specially adapted, individualized conditions and procedures" (Organizacija za ekonomsku kooperaciju i razvoj, 2007). In B&H in the last few decades, following terms are generally accepted: for persons under 18 years "children with disabilities", and for those above 18 years "people with disabilities" (Nuić, Kafedžić, Zejnilagić-Hajrić, 2013).

Teaching chemistry to students with disabilities is still not sufficiently explored. Opportunities for students with

visual or hearing impairments to participate in chemistry instruction are diverse and depend on a number of factors. Most important is the heterogeneity of this group of students. From the medical point of view, even though visual or hearing impairments do not entail other forms of damage and disorder (intellectual, cognitive, etc.), they are quite often combined. Other important factors are suitability of classrooms and teaching methods. This particularly relates to whether they are educated in special schools for visually/hearing impaired students, or they are included in regular schools. The school environment, teachers, methods and approaches, textbooks, instructional supplies and technology are factors that need special attention and which should be adapted to students with visual or hearing impairments.

Approximately 11% of school-age children in USA have some form of disability (Norman, Caseau and Stefanich, 1998). According to the Law for Primary and Secondary Education in B&H, children and young people with disabilities can gain an education in regular primary and secondary schools with an adapted curriculum, or in special educational institutions in those cases where it is impossible to provide adequate education in regular schools (DUGA, 2006; Organizacija za ekonomsku kooperaciju i razvoj, 2007; Dmitrović, 2011). According to information available on website of the Ministry of Education, Science and Youth of Canton Sarajevo, total number of children with disabilities in 35 regular secondary schools in Canton Sarajevo, in 2012/2013 school year was 877: most children with behavioral problems (318) and children with chronic illnesses and physical impairments (292), followed by children with intellectual disabilities (166). There also were children with combined (57) and speech disturbances (44), children with visual (29) and hearing impairments (21) (Izvještaj nevladinih organizacija, 2013). Only four schools have adapted interior area designed for students with disabilities; only one school provides information on Braille and sound signalization.

There are many reasons why inclusive education in B&H is not as present as it could and should be. It requires the full adaptation of curricula, schools, forms and methods of teaching. However, the biggest problem in the inclusion of children with disabilities in regular schools is lack of awareness on this subject as well as a lack of willingness by educational personnel (and government in general) to apply inclusion. Inclusion implies involvement of students with disabilities into regular classes and provides opportunities for them to participate in activities that modern school requires (Dmitrović, 2011). However, inclusion is also referred to students who do not have disabilities, but also learn how to perceive individual differences among their friends and classmates.

Models and variants of individualized teaching, especially in dealing with below-average students and also with above-average students are often appropriate in inclusive teaching as well (Ilić, 2009).

This study points to the problem of education of students with visual or hearing impairments in B&H, especially in teaching chemistry, as well as the problem of their enrollment to higher education institutions, such as Faculty of Science (Department of Chemistry).

RESEARCH METHODOLOGY

Participants

The study was conducted in school year 2013/14, in "Center for Blind and Visually Impaired Children and Youth (CSSDO)" and "Center for Hearing and Speech Rehabilitation (CSGR)", both located in Sarajevo. Total number of participants was 25 students: 11 students from CSSDO and 14 students from CSGR. The students who were tested are of high school age, attending different classes and orientations. Sample of 25 students is chosen based on teachers' and principals' recommendation, since students' disabilities are diverse and therefore they are all unique and there are different variables that affect their achievements.

Research hypotheses

H1- there is no statistically significant difference in knowledge of general chemistry between students of two Centers

H2- students from both Centers have the same probability for passing the "threshold" required for admission to the Faculty of Science, considering results of the test of knowledge, noting that they should have good results and grades in chemistry during their education

Instruments for collecting data

In this study general chemistry knowledge test (GCKT) and a questionnaire were used. Both instruments were designed for the purpose of this research; they were identical, voluntary and anonymous for all participants.

GCKT contained 19 items related to the basic concepts and theories of general chemistry. Some items required filling the statement, or grouping terms according to their properties (for example, compounds and mixtures), multiple choice questions etc. Instruments were adequately adapted for visually and hearing impaired students.

Altogether students were able to gain a maximum of 22 points. Passing threshold was set to be 40% (8.8 points).

The questionnaire contained 19 questions partially based on Likert-type, whose aim was to get insight into attitudes of participants about their potential enrollment to higher education institutions such as the Faculty of Science (Department of Chemistry).

RESULTS AND DISCUSSION

GCKT

For items 1-6, 9, 14 and 18 students had to choose correct answer and were able to gain 0.5 points/question. Items 7, 16 and 17 required grouping given terms. Every correct answer was worth 2 points. Items 8, 10-13, 15 and 19 required inserting adequate words in the sentence.

The percentage of CSSDO and CSGR students who completely answered given questions are presented in Figure 1.

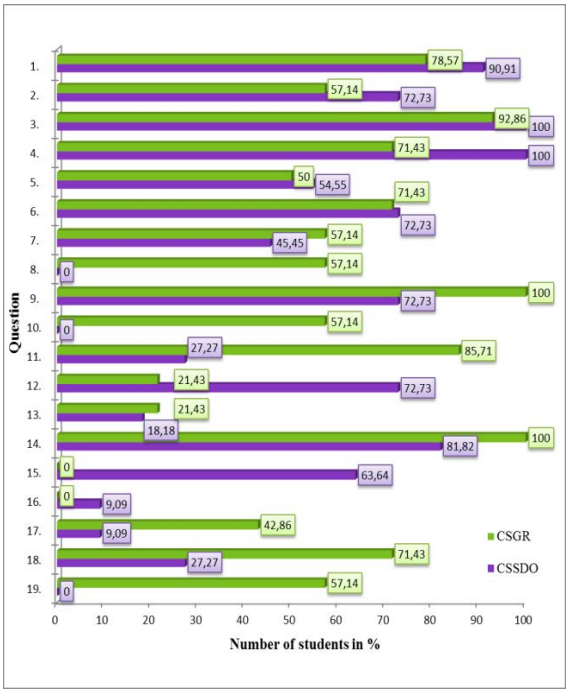


Figure 1: Comparison of CSSDO and CSGR students' correct answers on GCKT

Only on the 3rd (unit for amount of substance) and 4th (valence of hydrogen) item all CSSDO students gave correct answer. On the other side, all CSGR students have answered correctly only the 9th (location of metals in PTE) and 14th (concept of isotope) question.

Following items did not result in maximum number of correct answers: 8th (number of protons, electrons and neutrons in given example), 10th (charge of the given particle) and 19th (valence shell and valence electron).Interesting fact is correct answer of all CSSDO students on the question about valence of hydrogen, but at the same time they didn't know the meaning of valence theory. Unlike the CSSDO, CSGR students did not gain maximum points on only two questions: 15th (regarding reactants and products of chemical reaction) and the 16th (regarding classification of substances according to polarity).

Comparison of achieved points in GCKT is presented in Figure 2.

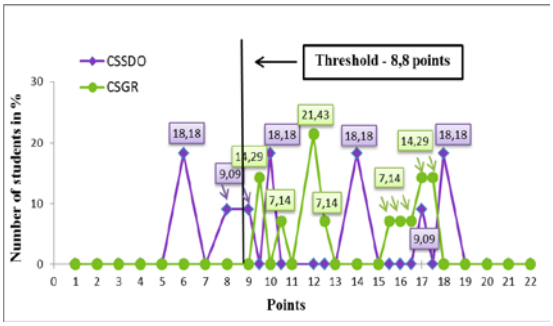


Figure 2: Comparison of students' achievements on GCKT

CSGR students gained slightly better results than CSSDO students. However, none of tested students have gained maximum score of 22 points; also there were no students with zero points. Highest score CSSDO students was 18 points (18.18%), while the highest score of CSGR students was 17.5 points(14.29 %).

Threshold was set to be 40%, or 8.8 points. More than half (72.73%) CSSDO students and all CSGR students passed the threshold. The lowest score of CSSDO students was 6 points (18.18%), while the lowest score of CSGR students is 9.5 points (14.29%).

The value of calculated t-test was $t = 2.11$. For the significance of 0.05 and confidence within the 95% theoretical t-test is $t = 2.11$, and for significance of 0.01 and confidence within the 99% theoretical $t = 2.90$. Based on these results first hypothesis can be partially accepted.

Questionnaire

In the first part of questionnaire, students were asked to give certain personal information about them.

In the second part of the questionnaire we wanted to found out the general attitude of the participants towards chemistry as a school subject using the Likert-type scale questions: (1-completely disagree; 3-partially agree; 5-completely agree).

In the third part, students were expected to offer a short and honest answer to questions also related to chemistry as a school subject, in addition to three issues related to the chemical industry and chemical products as well as how they imagine the job of a chemists.

Fourth part of the questionnaire was aimed to give information whether surveyed students with visual or hearing impairments would like to study in general and whether they are interested in studying chemistry. At the end of the questionnaire there was a possibility for students to write a comment.

The questionnaire revealed that students of both Centers consider chemistry as a difficult school subject. They said they have problems with learning and understanding chemistry and that they need more time for learning chemistry than some other school subjects. Many of these students do not want more hours of chemistry per week than prescribed by Curriculum.

Majority of students in both Centers do not doubt the importance of chemistry in everyday life, but certain percentage of disagreement gives a dose of concern. This may indicate that some students do not know how to link theory to everyday life and that they probably have never thought about importance of chemistry or that chemistry is present in their everyday lives, other than merely as a school subject.

Majority of CSSDO students, when asked if there is something to change about school subject chemistry wrote "How teacher teaches" or just "The teacher." The rest of the students wrote that they would reduce the theory they need to learn and bring more experiments and practical work. This may indicate that they are not satisfied with the methods and forms of work that the teacher uses. Teachers' choice of methods and types of teaching depends on the conditions in educational institution or in the chemistry classroom.

In teaching students with any kind of disability it is necessary to use adapted teaching aids. If the educational institution is unable to provide them, it is expected that a teacher cannot fully satisfy the demands of modern education of students with visual impairments. Encouraging is the fact that students are aware of the importance of practical work in teaching chemistry and

that regardless of the their disability they want to experience as much of this practical work as they can.
Some of CSGR students also stated they would like to reduce the theory and to bring more experiments. One student said that he would like to visit different factories or to go to some other kind of school trip. It should again be emphasized that educational institution should provide adequate laboratory equipment and supplies, but also the teacher needs to be creative.
Probably the most important questions for this study were: "What do you intend to do after graduating high school?" and "Would you like to study chemistry (at university level)?".Students' answers can be seen on Fig 3 and Fig. 4.

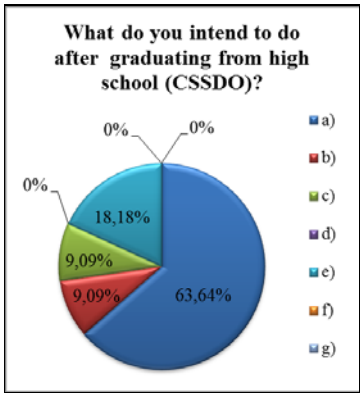


Figure 3: Answers of CSSDO students

(Legend: **a)** I intend to go to university (specified field); **b)** I intend to go to university but I still haven't decide what; **c)** I intend to look for a job in the profession I was educated in; **d**) I intend to look for a job but not in the profession for I was educated in; **e)** I intend to go on further training (courses etc.); **f)** I haven't yet decided; **g)** Other (please specify)

Figure 3 shows that 72.73% of CSSDO students want to continue their education at one of the higher education institutions and 63.64% students even know which faculty they wish to go to. A very low percentage of 9.09% of these students want to look for a job and work in their profession and 18.18% want to go on further training and courses.

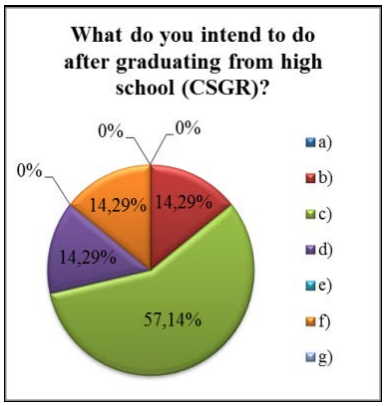


Figure 4: Answers of CSGR students.

Legend: **a)** I intend to go to university (specified field); **b)** I intend to go to university, but I do haven't decide what; **c)** I intend to get a job in the profession for which I was educated; **d)** I intend to get a job but not in the profession for which I was educated; **e)** I intend to go on further train (courses etc.); **f)** I haven't yet decided; **g)** Other (please specify)

Figure 4 shows that 57.14% CSGR students would like to work in the profession they are educated in and 14.29% students wish to work but not in their profession. The same number of these students has not yet decided. An alarming fact is that only 14.29% of CSGR students want to study at university level, but none of them yet decided what. This indicates that the students are not interested in further education or perhaps they are discouraged by the current situation in B&H regarding education of persons with disabilities at higher education institutions

Students' answers to the question "Would you like to study chemistry?" can be seen in Fig. 5 and Fig. 6.

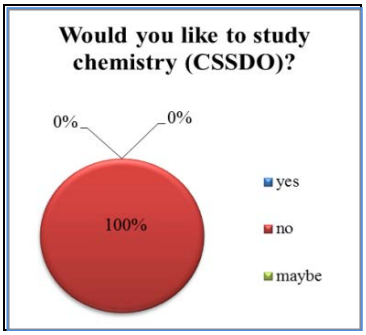


Figure 5: Answers of CSSDO students on question "Would you like to study chemistry?"

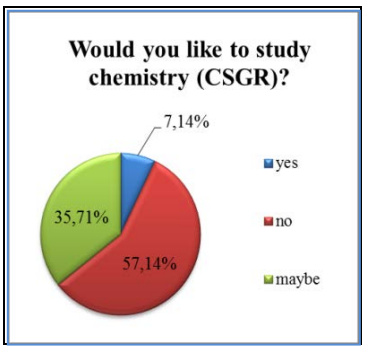


Figure 6: Answers of CSGR students on question "Would you like to study chemistry?"

None of CSSDO students would like to study chemistry at university level. Most of these students wrote they are not interested in science, or that chemistry as a school subject is too difficult and complicated. Devastating fact is that half of CSSDO students wrote that they would not be able to study chemistry because they have vision impairment. Obviously, these students are not familiar with the possibilities offered to them in modern chemistry education, but also in modern chemistry as a science, and that there are many blind and low-vision scientists who have very successful careers as a chemists and in other professions related to science.
From the Figure 6.it can be seen that most CSGR students (57.14%) would not like to study chemistry at university level, 35.71% students would consider this option and 7.14% surveyed students would like to study chemistry. Most of the students have not explained their answer. However, one student wrote that he would be interested for job as a forensic scientist. Only two CSGR

students are not interested in chemistry. It is interesting that none of surveyed CSGR students has noted his/hers impairment as a reason for not studying chemistry.

CONCLUSIONS

Results of the study showed:

(1) There is no significant difference in students' achievements on knowledge test in general chemistry (GC) in CSSDO and CSGR;

(2) Considering their achievements in GC, they have a chance to enroll university study of chemistry based on earlier entrance exams;

(3) Majority of students would like to enroll to university after secondary school;

(4) Only one student would consider studying chemistry.

Opportunities for students with visual or hearing impairments to participate in chemistry class are diverse and depend on a number of factors. There are ways to "bring chemistry" and to adapt teaching methods and supplies for teaching students with disabilities, where teachers' role is especially emphasized. The role of the teacher in teaching process is irreplaceable, but her/his role in teaching students with disabilities has another dimension because there are additional factors that need to be cared for. Creativity of chemistry teacher is particularly evident in teaching students with disabilities – in addition to the knowledge of chemistry and education of chemistry, a solid knowledge on specifics of teaching chemistry to students with disabilities is needed. However, due to many problems, students with disabilities are rarely enrolled to university courses. Students with disabilities are often demoralized during their education and this can be one of the reasons that they do not sufficiently attempt to enroll on higher education institutions.

REFERENCES

Dmitrović, P. (2011). Preduvjeti za primjenu inkluzije, *Metodički obzori 13*, 6 (3), 69-82

Društvo ujedinjenih građanskih akcija-DUGA. (2006). *Vodič kroz inkluziju u obrazovanju.* Sarajevo: Udruženje DUGA. http://www.monkstk.ba/attachments/article/115/Prirucnik%20Uvod%20u%20inkluziju.pdf [03.12.2013]

Društvo ujedinjenih građanskih akcija-DUGA. (2013). *Smjernice za školovanje djece s posebnim potrebama u srednjim školama Bosni i Hercegovini*. Sarajevo: Udruženje DUGA. http://www.fmon.gov.ba [03.12.2013]

Ilić, M. (2009). *Inkluzivna nastava*. Istočno Sarajevo: Filozofski fakultet Univerziteta u Istočnom Sarajevu.

Izvještaj nevladinih organizacija (2013). *Primjena Konvencije o pravima osoba sa invaliditetom u Bosni I Hercegovini*. http://www.rightsforall.ba/publikacije-bs/docs-bs/izvjestaj-nevladinih-organizacija.pdf [17.06.2013]

Miner, D.L., Nieman, R., Swanson, A.B, Woods, M. (Eds.) (2001).Teaching Chemistry to Students with Disabilities: A Manual for High Schools, Colleges, and Graduate Programs. 4th Edition. Washington, DC: American Chemical Society. Available at https://www.acs.org/content/dam/acsorg/education/publications/teaching-chemistry-to-students-with-disabilities.pdf [05.12.2013.]

Norman, K., Caseau, D., Stefanich, G.P. (1998). Teaching Students with Disabilities in Inclusive Science Classrooms: Survey Results. Science Education 82, 127–146.

Nuić, I., Kafedžić, L., Zejnilagić-Hajrić M. (2013). Mogućnosti u nastavi kemije za učenike s teškoćama u razvoju. *Didaktički putokazi* 19 (65), 80-85

Organizacija za ekonomsku kooperaciju i razvoj. (2007). *Obrazovna politika za rizične učenike i učenike s teškoćama u Jugo-Istočnoj Evropi - Bosna i Hercegovina.* Pariz: OECD. http://www.oecd.org/edu/innovation-education/38614112.pdf [03.12.2013]

Summary/Sažetak

Obrazovanje učenika s teškoćama u razvoju u Bosni i Hercegovini regulirano je Zakonom o osnovnom i srednjem obrazovanju odgovarajućih institucija (ministarstava). Obrazovanje se može provoditi u redovnim školama sa ili bez prilagođenog nastavnog plana i programa, te u specijalnim centrima. U ovom radu prikazani su rezultati istraživanja provedenog u Centru za slijepu i slabovidnu djecu i omladinu (CSSDO) i u Centru za slušnu i govornu rehabilitaciju (CSGR). Cilj istraživanja bio je ispitati mogućnosti i interes učenika za studiranje hemije na fakultetu. Rezultati su pokazali: (1) ne postoji statistički značajna razlika u postignućima učenika ova dva centra na testu znanja iz opće hemije, (2) prema rezultatima testa znanja, postoji mogućnost njihovog upisivanja na studij hemije, prema ranijim kriterijima polaganja prijemnog ispita, (3) većina učenika bi se voljela upisati na fakultet, (4) ali samo jedan učenik bi razmatrao studiranje hemije. Ovo istraživanje ukazuje na problem uključivanja učenika sa oštećenjem vida ili sluha u studijske programe prirodnih nauka na fakultetima, a također i nedostatak potrebnog obrazovanja za nastavno osoblje na fakultetima i u srednjim školama kada se radi o obrazovanju učenikas teškoćama u razvoju.

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2015

44

71-78

Print ISSN: 0367-4444
Online ISSN: 2232-7266

UDC: _____

Original Scientific Article

Pigments and genome size variation in *Symphyandra hofmannii* population

Parić, A.[a], Karalija, E.[a], Pustahija, F.[a,b,c], Šiljak-Yakovlev, S.[a,c], Muratović, E.[a*]

[a]*University of Sarajevo, Faculty of Sciences, Department of Biology, Zmaja od Bosne 35, 71000 Sarajevo, B&H*
[b] *University of Sarajevo, Faculty of Forestry, Zagrebačka 20,71000 Sarajevo, B&H*
[C] *Laboratoire Ecologie, Systématique, Evolution, UMR 8079, CNRS-UPS AgroParisTech, Université Paris-Sud, Bât. 360, 91405 Orsay, France*

Article info
Received: day/month/year
Accepted: 29/6/2015

Keywords:
Campanulaceae,
genome size,
pigments,
Symphyandra hofmannii

***Corresponding author:**
E-mail:
edina.muratovic@pmf.unsa.ba
Phone: + 387 33 723 725
Fax: 00-000-00-0000000

Abstract: The photosynthetic pigments, total anthocyanin content and genome size in six natural populations of endemic species *Symphyandra hofmannii* Pant. (Hoffman's ring bellflower) from Bosnia and Herzegovina were investigated in the present study. The intrapopulation and interpopulation analysis of *Symphyandra hofmannii* growing under different environmental conditions were achieved. The interpopulation analysis of individuals developed under different environmental conditions had shown statistically significant differences for all investigated parameters (chlorophyll *a* and *b*, total chlorophyll, chlorophyll *a*/*b* ratio, carotenoids, anthocyanins and DNA amount). Statistically significant differences between individuals from the same population and different habitats, in chlorophyll *b*, *a/b* ratio and anthocyanins content, were also apparent. Interpopulation pigments' variation could not be related to light as only differential ecological parameter. This variation was depended on combined environmental conditions, such as geological substrate, altitude or anthropogenic factors. The genome size was significantly higher only for one serpentine population (Papratnica).

INTRODUCTION

At present, genus *Symphyandra* A. DC, family Campanulaceae, is represented in Europe by three species (Tutin, 1996): *S. cretica* A. DC distributed in Greece, *S. wanneri* (Rochel) Heuffel in mountains of Bulgaria, Romania and eastern part of ex Yugoslavia and *S. hofmannii* Pant. in Bosnia and Herzegovina.

Symphyandra hofmannii (Hoffman's ring bellflower) was discovered by botanist Sendtner in 1842 and described as *S. wanneri* (Rochel) Heuffel. Four decades later Pantocsek (1881) recognized it as a separate species (Pantocsek, 1881; Fukarek, 1956; Tutin, 1996). Endemic for central Dinaric Alps, this species is distributed in the central part of Bosnia and Herzegovina. Present distribution is restricted to river basins Bosna and Vrbas, including few isolated populations along the river Tinja.

The most of the data about *S. hofmannii* concern morphological (Malý, 1948; Slavnić, 1966), chorological or ecological characteristics (Malý, 1948; Fukarek, 1956; Redžić, 1976). It is on the list for the future "Red book" of Bosnia and Herzegovina described as a rare species (Šilić, 1996, Đug et al. 2013).

Symphyandra hofmannii is biennial species with basal leaves grouped in a rosette and pale yellow pending flowers. Species' morphological features could be distinguished according to different environmental conditions. Short individuals (up to 30 cm) with smaller flowers, including lanceolate, dark green leaves are associated with semi humid and heliophilous habitats. Whereas, tall (up to 50 cm), robust individuals with bigger flowers and light green ovate leaves inhabit humid and sciophilous environments (Slavnić, 1966).

According to different authors *S. hofmannii* is eurivalent species characterized by different environmental conditions (Malý, 1948; Fukarek, 1956; Slavnić, 1966; Redžić, 1976; Šilić, 1990). Its populations can be found at different altitudes (range from 140 m to 900 m),

inclinations, geological substrates (limestone, silicate, serpentine, gabbro, malachite, shale clay), and on shallow or deep soils. This species is also adaptable to different intensity of solar radiation. According to Slavnić (1966), the only common habitat characteristic for all populations is air or soil humidity.

Chlorophyll a and b contents and their ratio have very important role in adaptation of photosynthetic apparatus of higher plants on different light regimes (Porra, 2002). Such analysis can be crucial for investigation of plant's ecological adaptations. In addition, the carotenoids are also associated with the photosynthetic apparatus and their content variation could be an answer to different light regimes as well. Although light intensity was thought as most important factor affecting plant chlorophyll content (Porra, 2002), some other environmental factors could also be included, such as altitude, slope, vegetation density (Boquera et al., 2010), temperature, water stress (Bokhari, 1976) and mineral nutrition (Bokhari, 1976; Bojović and Stojanović, 2005).

High light exposure of plants often results in anthocyanins accumulation in leaves. It is supposed that anthocyanins could adjust the amount of the light passing through the leaf and in that way prevent photoinhibition (Gould et al., 1995; Burger and Edwards, 1996).

DNA amount is also one of the biodiversity characters with various practical implications (Bennett and Leitch, 2005). Although the genome size thought to be constant at species rank, there are frequent reports of intraspecific variation (Blondon et al., 1994; Reeves et al., 1998; Moscone et al., 2003). Moreover, recent investigations show the increasing interest for association of genome size variation with geographic and ecological factors (Slovak et al., 2009), that frequently includes detection of differences in DNA content (Doležel, 1991; Price and Johnston, 1996; Doležel and Bartoš, 2005; Siljak-Yakovlev et al., 2008; Muratović et al., 2010; Siljak-Yakovlev et al., 2010; Pustahija et al., 2013).

In the present paper pigments contents and genome sizes were compared among six populations of *Symphyandra hofmannii* with different habitat conditions. This study was particularly focused on inter/intrapopulation variation of studied parameters, as possible consequence of adaptation to different environmental conditions.

EXPERIMENTAL

Plant material

Flowering plants were collected from six *S. hofmannii* natural populations. All specimens from investigated populations were collected at once during the same day in the morning hours. Temperature ranged from 18 to 21 °C for all localities. Investigated populations are characterized by contrasting ecological conditions (e.g. different geological substrate, soil, altitude, and habitat type). All analyses were performed on the same individuals. Within two investigated populations different type of habitat were detected and their specimens are separately analyzed: Bistričak (fringe zone of mixed forest and lowland hay meadow) and Vitovlje (fringe zone of mixed forest and rocky slopes). Provenance of investigated populations, their basic geographic and habitat characteristics are shown in Table 1.

Fresh leaves (from the central part of stem) from 10 individuals per population were collected for chlorophyll a and b and carotenoids contents analysis. To prevent chlorophyll pheophytinization, CaCO3 was added during the leaves sampling. The extractions and analyses were done in the same day. Air-dried lives, from at least two individuals per population were used for anthocyanin determinations. Fresh leaves from at least five individuals from each population were analyzed to estimate the genome size. Vouchers are deposited in the Laboratory for Research and Protection of Endemic Resources, Faculty of Sciences, University of Sarajevo.

Pigments determination

Photosynthetic pigments content: Photosynthetic pigments content was determined spectrophotometrically according to Holm (1954) and Porra et al. (1989). Chlorophylls a and b and carotenoids content was determined by absorbance reading at 663, 645 and 440 nm, respectively, and calculated as mg pigment per g of fresh weight. All experiments were performed in three replicates per sample. Total anthocyanin content: Total anthocyanins were determined using the pH differential method according to Giusti and Wrolstad (2001). Disposable cuvettes were used for spectral measurements at 530 and 700 nm. Pigment content was calculated as cyanidin 3-glucoside (cyd 3-glc), using an extinction coefficient of 26 900 L cm–1 mol–1 and molecular weight of 448.8g/mol All experiments were performed in three replicates per sample.

Flow cytometry

Plant material for flow cytometry was prepared according to Marie and Brown (1993). DNA content of 5000-10000 stained nuclei was determined for each sample using a Flow Cytometer SL3 (Partec, Münster, Germany). *Petunia hybrida* PxPc6 (2C = 2.85 pg) was used as an internal standard. Total 2C DNA value was calculated using the linear relationship between the fluorescent signals from stained nuclei of the unknown specimen and the known internal standard.

Statistical analyses

Data was analyzed by Statistica 7 for Windows, using a one-way ANOVA. The differences were tested using significance at $p<0.01$ and $p<0.05$. To determine the significant differences between group means, after analysis of variance, post hoc Newman-Keuls test was performed.

RESULTS

Effects of the different environmental conditions on some physiological parameters and DNA content in *Symhyandra hofmannii*, an endemic and eurivalent species, were studied. Results of pigments and genome size measurements for investigated species *Symphyandra hofmannii* are presented in the Table 2. Detected variation of these investigated parameters in *S. hofmannii* populations showed different response to diverse environmental conditions.

Interpopulation analysis, considering different life settings (Table 1), showed significantly increased level of chlorophyll a for Papratnica population, as well as significant increase in DNA content at 5% level. Papratnica also had the highest total chlorophylls comparing to the content found in other populations.
Carotenoid content showed statistically significant differences between Papratnica (highest detected value) and Donji Vakuf (lowest detected value), with no significant differences comparing to other populations (Table 2).
Significant differences were also found for chlorophyll b, chlorophyll ratio and anthocyanins (Table 2). The anthocyanins were not detected in all investigated populations (Table 2).

Table 1: Origin of investigated *Symphyandra hofmannii* populations (1– not directly exposed to light, 2 – directly exposed to light)

Localities	Latitude	Longitude	Altitude (m)	Geological substrate	Type of habitat
Bistričak	44°21´16´´ N	17°58´40´´ E	451	Chert, silicate limestone	Fringe zone of mixed forest (1)/lowlend hay meadow (2)
Jajce	44°20´30´´ N	17°16´00´´ E	404	Tufa, limestone conglomerates	Fringe zone of mixed forests near human settlement (1)
Vitovlje	44°20´45´´ N	17°30´04´´ E	841	Limestone shallow soils	Fringe zone of mixed forest (1)/rocky slopes (2)
Vinac	44°16´38´´ N	17°30´04´´ E	397	Limestone	Rocky crevices (2)
Papratnica	44°26´14´´ N	17°58´40´´ E	333	Serpentine	Fringe zone of mixed forest (1)
Donji Vakuf	44°12´29´´ N	17°19´56´´ E	481	Slate, aleurolite	Rocky slopes (2)

Intrapopulation study included only two populations with different types of habitat related to the direct exposure to light or not, Bistričak (fringe zone of mixed forest and lowland hay meadow) and Vitovlje (fringe zone of mixed forest and rocky slopes) (Table 1). Intrapopulation analysis of genome size was excluded from this study, since no significant intrapopulation differences were found. No significant differences among individuals from different types of habitat within one population for chlorophyll *a*, total chlorophylls and carotenoid content were found (Table2). Significant differences were only detected for chlorophyll *b*, *a/b* ratio and anthocyanins content (Table 2).

Table 2. Pigments composition and DNA amount for investigated *Symphyandra hofmannii* populations

Populations	Bistričak		Jajce	Vitovlje		Vinac	Papratnica	Donji Vakuf
Light	1	2	1	1	2	2	1	2
Chlorophyll *a* (mg g^{-1} ±S.D.)	0.12^{b} (±0.05)	0.13^{b} (±0.12)	0.15^{b} (±0.03)	0.09^{b} (±0.02)	0.12^{b} (±0.02)	0.15^{b} (±0.06)	0.21^{a} (±0.06)	0.11^{b} (±0.03)
Chlorophyll *b* (mg g^{-1} ±S.D.)	0.10^{bc} (±0.05)	0.07^{cd} (±0.05)	0.15^{a} (±0.04)	0.11^{b} (±0.02)	0.05^{d} (±0.01)	0.07^{cd} (±0.03)	0.12^{ab} (±0.03)	0.06^{d} (±0.02)
Chlorophyll (*a*+*b*) (mg g^{-1} ±S.D.)	0.27^{b} (±0.11)	0.26^{b} (±0.22)	0.34^{ab} (±0.08)	0.22^{b} (±0.04)	0.23^{b} (±0.03)	0.29^{b} (±0.11)	0.42^{a} (±0.11)	0.22^{b} (±0.06)
a/b ratio	1.20^{c} (±0.06)	1.77^{b} (±0.02)	1.01^{cd} (±0.13)	0.87^{d} (±0.10)	2.35^{a} (±0.39)	2.17^{a} (±0.23)	1.71^{b} (±0.17)	1.82^{b} (±0.06)
Carotenoids (mg g^{-1} ±S.D.)	0.46^{ab} (±0.12)	0.42^{ab} (±0.32)	0.54^{ab} (±0.12)	0.43^{ab} (±0.11)	0.42^{ab} (±0.02)	0.46^{ab} (±0.16)	0.61^{a} (±0.14)	0.38^{b} (±0.09)
Anthocyanins (mg L^{-1} ±S.D.)	0.19^{e} (±0.02)	1.34^{c} (±0.14)	0.23^{e} (±0.16)	0.58^{d} (±0.12)	2.27^{b} (±0.45)	2.65^{a} (±0.41)	nd	0.16^{e} (±0.02)
DNA amount (pg ±S.D.)	3.76^{b} (±0.03)		3.83^{b} (±0.04)	3.84^{b} (±0.02)		3.83^{b} (±0.03)	3.94^{a} (±0.02)	3.78^{b} (±0.01)

1 – individuals/population not directly exposed to light; 2 – directly exposed to light; (±S.D.) – data represents average values; nd – not detected; individuals/populations not shearing the same letter within one parameter differ significantly at $p \leq 0.05$ level.

DISCUSSION

Investigated variation of pigments in *Symphyandra hofmannii* showed different response to diverse environmental conditions. Although many authors confirmed that chlorophyll content was higher in leaves directly exposed to sun instead to those from shade (Castrillo et al., 2001; Medvegy et al., 2005), opposite findings also exist (Johnson et al., 1982; Sarracino et al., 1992; Chartzoulakis et al., 1995; Souza and Válio, 2003; Dias et al., 2007). There is still no consensus about increasing or decreasing of chlorophyll *a* and *b*, total chlorophylls, carotenoids or chlorophyll *a*/*b* ratio in correlation to different light conditions (Johnson et al., 1982; Sarracino et al., 1992; Chartzoulakis et al., 1995; Souza and Válio, 2003; Dias et al., 2007). This study showed that direct exposure to light (Populations Bistričak 2, Vitovlje 2, Vinac 2 and Donji Vakuf 2) is not exclusively the cause of the chlorophyll content increase, however other ecological factors also have their influence. For instance, population Papratnica had the highest value of chlorophyll *a*, total chlorophylls and carotenoids due to the stressful conditions caused by serpentine geological substrate. Furthermore, increasing of chlorophyll content under stress conditions was reported by different authors (Jamil et al., 2007; Pinheiro et al., 2008; Mafakheri et al., 2010; Rahdari et al., 2012).

Investigated parameter chlorophyll *a/b* ratio gave clearer picture where its increase is significantly associated with direct exposure to light in populations Vitovlje 2 and Vinac, followed by Bistričak 2 and Donji Vakuf, with exception of population Papratnica. Despite the fact that decrease of chlorophyll *a/b* ratio is related to drop in light intensity (Beneragama and Goto, 2010; Biswal et al., 2012) our results showed that it might not be always a rule, especially when stressful conditions such as serpentine substrate are implicated (Table 2).

Although the anthocyanins were not identified in all investigated populations (Table 2), significantly higher anthocyanins values were always found in populations/individuals that were directly exposed to the light, with exception of population Donji Vakuf. Absence of anthocyanins in Papratnica population characterized by extremely dense zone of mixed forest directly showed dependence of their concentration to light intensity. Contrary to this, very low concentration of anthocyanins content in open habitats, like recorded in D. Vakuf suggesting that some other factor can affect anthocyanins content (Neufeld et al., 2011)

The genome size was significantly higher only for serpentine population Papratnica (Table 2). Possible explanation for recorded increase of DNA amount is a result either of B chromosomes presence or increasing of GC bands of individuals in serpentine substrates (Pustahija et al., 2013). Namely, serpentine soils produce extreme ecological conditions for plant development (Riter-Studnicka, 1963; Kruckeberg, 1984; Proctor and Nagy, 1992; Baker et al., 1993; Brady et al., 2005). These soils contain low concentration of plant nutrients, low Ca/Mg ratio and high concentration of heavy metals: Cr, Ni, Co and Mn (Kruckeberg, 1992; Proctor, 1999; Oze et al., 2004). In addition, serpentine soils are usually shallow and permeable for water, which induced for *S. hofmannii* unfavorable dry habitats. But, this population exists in habitat with higher aerial humidity which, probably, allowed its surviving on serpentine soil. *Symphyandra hofmannii* is known as eurivalent species for different habitats (Malý, 1948; Fukarek, 1956; Slavnić, 1966; Redžić, 1976; Šilić, 1990) with air or soil humidity as the only common characteristic (Slavnić, 1966). Additionally, it is known that plant adaptations on such atypical geological substrates can be recognized as individual morphological and physiological changes (Riter-Studnicka, 1963; Iturralde, 2001; Muratović et al., 2005). Moreover, the collected individuals from Papratnica population differed in their size from all others populations. Instead to be up to 50 cm in height, what is a maximum for humid and habitats not directly exposed to the light (Slavnić, 1966), these individuals were over 100 cm high.

Intrapopulation investigation of *S. hofmannii* included two populations, Bistričak and Vitovlje (Tables 1 and 2). This analysis (where all other ecological factors except exposure to the light were excluded) confirmed that higher light intensity is not solely in positive correlation with chlorophyll content. Therefore, no significant differences among individuals from different light regimes within one population for chlorophyll *a*, total chlorophylls and carotenoid content were found (Table 2).

Our results confirmed that from all analyzed parameters only chlorophyll *b*, *a/b* ratio and anthocyanins content were directly related to light regime (Table 2). When other ecological factors are included these findings could deviate and such results might give an explanation for the opposite findings by different authors (Johnson et al., 1982; Sarracino et al., 1992; Chartzoulakis et al., 1995; Castrillo et al., 2001; Souza and Válio, 2003; Medvegy et al., 2005; Dias et al., 2007).

Interpopulation analyses gave no clear picture about the effect of exposure to the light since exclusion of other ecological factors was not possible. Such findings confirmed that intrapopulation analyses are unavoidable part of investigation dealing with the effect of light regimes on photosynthetic pigments. Intrapopulation analyses clarified that chlorophyll *b*, *a/b* ratio and anthocyanin content are the only two parameters directly dependent upon light regime. Given that *S. hofmannii* is rare, endemic but also very attractive horticultural species, further analysis of its photosynthetic light response, leaf nitrogen content, stomata conductance, leaf area index and other anatomical features should be enlightening for its planting and expansion.

ACKNOWLEDGEMENTS

The authors thankfully acknowledge to S. Brown and O. Catrice (Institut des Sciences du Végétal, UPR 2355, CNRS, Gif-sur-Yvette, France) for collaboration in nuclear DNA content assessment.

REFERENCES

Baker, A.J.M., Proctor, J., Reeves, R.D. (1993). *The Vegetation of Ultramafic (Serpentine) Soils*. Intercept Ltd., Andover, UK.

Beneragama, C.K., Goto, K. (2010). Chlorophyll a:b ratio increases under low-light in 'shade-tolerant' *Euglena gracilis*. *Tropical Agricultural Research, 22*,12-25.

Bennett, M., Leitch, I.J. (2005). Nuclear DNA amounts in angiosperms – progress, problems and prospects. *Annals of Botany, 95*, 45-90.

Biswal, A.K., Pattanayak, G.K., Pandey, S.S., Leelavathi, S., Reddy, V.S., Govindjee, Tripathy BC. (2012). Light intensity-dependent modulation of chlorophyll *b* biosynthesis and photosynthesis by overexpression of chlorophyllide *a* oxygenase in tobacco. *Plant Physiology, 159*, 433-449.

Blondon, F., Marie, D., Brown S., Kondorosi, A. 1994. Genome size and base composition in *Medicago sativa* and *M. truncatula* species. *Genome, 37*, 264-270.

Bojović, B., Stojanović, J. 2005. Chlorophyll and carotenoid content in wheat cultivars as a function of mineral nutrition. *Archives of Biological Sciences Belgrade, 57*, 283-290.

Bokhari, U.G. 1976. Influence of temperatures, water stress, and nitrogen treatments on chlorophyll and dry matter of western wheatgrass. *Journal of Range Management, 2*, 127-131.

Boquera, M.L.E., Morales, Ph.L.V.C. (2010). Leaf chlorophyll content estimation in the monarch butterfly biosphere reserve. *Revista Fitotecnia Mexicana, 33*, 175-181.

Brady, K.U., Kruckeberg, A.R., Bradshaw, Jr.H.D. (2005). Evolutionary ecology of plant adaptation to serpentine soils. *Annual Review of Ecology, Evolution, and Systematics,36*, 243-266.

Burger, J., Edward,s G.E. (1996). Photosynthetic efficiency, and photodamage by UV and visible radiation, in red versus green leaf *Coleus* varieties. *Plant Cell Physiology, 37*, 395-399.

Castrillo, M., Vizcaíno, D., Moreno, E., Latorraca, Z. (2001). Chlorophyll content in some cultivated and wild species of the family Lamiaceae. *Biologia Plantarum, 44*, 423-425.

Chartzoulakis, K., Therios, I., Noitsakis, B. (1995). Effects of shading on gas exchange specific leaf weight and chlorophyll content in four kiwifruit cultivars under field conditions. J*ournal of Horticultural Science, 68*,605-611.

Dias, J., Pimenta, J.A., Medri, M.E., Torres Boeger, M.R., Toledo de Freitas, C. (2007). Physiological aspects of sun and shade leaves of *Lithraea molleoides* (Vell.) Engl. (Anacardiaceae). *Brazilian Archives of Biology and Technology, 50*, 91-99.

Doležel, J., Bartoš, J. (2005). Plant DNA flow cytometry and estimation of nuclear genome size. *Annals of Botany, 95*, 99-110.

Doležel, J. (1991). Flow cytometric analysis of nuclear DNA content in higher plants. *Phytochemical Analysis, 2*, 143-154.

Đug, S., Muratović, E., Dreško vić, N., Boškailo, A., Dudević, S. (2013). *Crvena lista flore Federacije Bosne i Hercegovine, knjiga 2*. (Ed. Federalno Federalno ministarstvo okoliša i turizma). http://www.fmoit.gov.ba/download/Crvena/20lista/20Flore/20FBiH.pdf.

Fukarek, P. (1956). Nova nalazišta bosanske zvončike (*Symphyandra hofmanni* Pantocz.). *Godišnjak Biološkog Instituta Sarajevo, 9(1-2),* 131-140 (in Bosnian).

Giusti, M.M., Wrolstad, R.E. (2001). Unit F1.2.1-13. Anthocyanins. Characterization and measurement with UV visible spectroscopy. In Wrolstad, R.E. (Ed.), *Current protocols in food analytical chemistry*. Wiley, New York.

Gould, K.S., Kuhn, D.N., Lee, D.W., Oberbauer, S.F.(1995). Why leaves are sometimes red. *Nature, 378*, 241-242.

Holm, G. (1954). Chlorophyll mutations in barley. *Acta Agriculturae Scandinavica, 4*, 457-471.

Iturralde, R.B. (2001). The influence of ultramafic soils on plants in Cuba. *South African Journal of Science, 97*, 510-514.

Jamil, M., Lee, D.B., Jung, K., Ashraf, M., Lee, S.H., Rha, E.S. (2007). Effect of salt stress on germination and early seedling growth of four vegetables species. *Journal of Central European Agriculture, 7*, 273-281.

Johnson, C.R., Nell, T.A., Rosenbaum, S.E. (1982). Influence of light intensity and drought stress on *Ficus benjamina* L. *Journal of the American Society for Horticultural Science, 107*, 252-255.

Kruckeberg, A.R. (1984). *California Serpentines: Flora, Vegetation, Geology, Soils and Management Problems*. Berkeley, University of California Press.

Kruckeberg, A.R. (1992). Plant life of western North American ultramafic. In Roberts, B.A., Proctor J. (Ed.), *The ecology of areas with serpentized rocks*, Dordrecht, Kluwer Academic Publishers, The Netherlands.

Mafakheri, A., Siosemardeh, A., Bahramnejad, B., Struik, P.C., Sohrabi, Y.(2010). Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. *Australian Journal of Crop Science, 4*, 580-585.

Malý, K. 1948. *Symphynadra hofmanni* Pant. Prilog poznavnju vrste. *Godišnjak Biološkog Instituta Sarajevo, I 4-6*, 19-24 (in Bosnian).

Marie, D., Brown, S.C. 1993. A cytometric exercise in plant DNA histograms, with 2C values for 70 species. *Biology of the Cell, 78*, 41-51.

Medvegy, A., Kálmán, K., Lehoczki, E. (2005). Photosynthetic acclimation to light of the three taxa of *Primula vulgaris* x *P. veris* hybrid zone in the Bakony mountains. *Acta Biologica Szegediensis, 49*, 157-159.

Moscone, E.A., Baranyi, M., Ebert, I., Greilhuber, J., Ehrendorfer, F., Hunziker, A.T. (2003). Analysis of nuclear DNA content in *Capsicum* (Solanaceae) by flow cytometry and Feulgen densitometry. *Annals of Botany, 92*, 21-29.

Muratović, E., Bogunić, F., Šoljan, D., Siljak-Yakovlev, S. (2005). Does *Lilium bosniacum* merit species rank? A classical and molecular-cytogenetic analysis. *Plant Systematics and Evolution, 252,* 97-109.

Muratovic, E., Hidalgo, O., Garnatje, T., Siljak-Yakovlev, S. (2010). Molecular phylogeny and genome size in European lilies (Genus *Lilium*, Liliaceae). *Advanced Science Letters*, 3, 180-189.

Neufeld, H.S., Poindexter, D.B., Murakami, P.F., Schaberg, P.G. (2011). Observations on the relationship between above- and below-ground anthocyanin production in *Galax urceolata* (Poir.) Brummitt growing in sun-exposed and shaded locations. *Castanea, 76,* 84-98.

Oze, C., Bird, D., Coleman, R., Fendorf, S. (2004). Chromium geochemistry in serpentinized ultramafic rocks and soils from the Francsician complex of California. *American Journal of Science, 304,* 67-102.

Pantocsek, J. (1881). Ueber bosnisch-hercegovinische pflanzen und aus dem comitate neutra in Ungarn. *Oesterreichische botanische Zeitschrift Wien, 31,* 347-351.

Pinheiro, H.A., Silva, J.V., Endres, L., Dos Santos, B.G. (2008). Leaf gas exchange, chloroplastic pigments and dry matter accumulation in castor bean (*Ricinus communis* L.) seedlings subjected to salt stress conditions. *Industrial Crops and Products, 27,* 385-392.

Porra, R.J., Thompson, W.A., Kriedemann, P.E. (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption. *Biochimica et Biophysica Acta, 975,* 384-394.

Porra, R.J. (2002). The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls *a* and *b*. *Photosynthesis Research, 73,* 149-156.

Price, H.J., Johnston, J.S. (1996). Influence of light on DNA content of *Helinathus annuus* Linnaeus. *Proceedings of the National Academy of Sciences of the United States of America, 93,* 11264-11267.

Proctor, J., Nagy, L. (1992). Ultramafic rocks and their vegetation: an overview. In Baker, A.J.M., Proctor, J., Reeves, R.D. (Ed.), *The vegetation of ultramafic (serpentine) soils*. Intercept Ltd, Andover, UK.

Proctor, J. 1999. Toxins, nutrient shortages and droughts: the serpentine challenge. *Trends in Ecology & Evolution,14,* 334-335.

Pustahija, F., Brown, S.C., Bogunić, F., Bašić, N., Muratović, E., Ollier, S., Hidalgo, O., Bourge, M., Stevanović, V., Siljak-Yakovlev, S. (2013). Small genomes dominate in plants growing on serpentine soils in West Balkans, an exhaustive study of 8 habitats covering 308 taxa. *Plant Soil, 373,* 427-453.

Rahdari, P., Tavakoli, S., Hosseini, S.M. (2012). Studying of salinity stress effect on germination, proline, sugar, protein, lipid and chlorophyll content in purslane (*Portulaca oleracea* L.) leaves. *Journal of Stress Physiology & Biochemistry, 8,* 182-193.

Redžić, S. (1976). O bosanskoj zvončiki. *Biološki List, 24,* 117-118 (in Bosnian).

Reeves, G., Francis, D., Davies, M.S., Rogers, H.J., Hodkinson, T.R. (1998). Genome size is negatively correlated with altitude in natural populations of *Dactylis glomerata*. *Annals of Botany, 82,* 99-105.

Riter-Studnicka, H. (1963). Biljni pokrov na serpentinama u Bosni. *Godišnjak Biološkog Instituta Univerzitet Sarajevo, XVI,* 91-205 (in Bosnian).

Sarracino, J.H., Merritt, R., Chin, C.K. (1992). Morphological and physiological characteristics of *Leea coccina rubra* in response to light flux. *HortScience, 27,* 400-403.

Šilić, Č. (1990). *Endemične biljke*. Zavod za udžbenike i nastavna sredstva Sarajevo, Zavod za udžbenike i nastavna sredstva, Beograd.

Šilić, Č. (1996). *Spisak biljnih vrsta (Pteridophyta i Spermatophyta) za Crvenu knjigu Bosne i Hercegovine.* GZM (PN) NS, Sarajevo, 31:323-367 (in Bosnian).

Siljak-Yakovlev, S., Pustahija, F., Solic, E.M., Bogunic, F., Muratovic, E., Basic, N., Catrice, O., Brown, C.S. (2010). Towards a database of genome size and chromosome number of Balkan flora: C-values in 343 taxa with novel values for 252. *Advanced Science Letters, 3,* 190-213.

Siljak-Yakovlev, S., Stevanovic, V., Tomasevic, M., Brown, S.C., Stevanovic, B. (2008). Genome size variation and polyploidy in the resurrection plant genus *Ramonda*: cytogeography of living fossils. *Environmental and Experimental Botany, 62,* 101-112.

Slavnić, T. (1966). O infraspecijskim oblicima bosanske zvončike (*Symphyandra hofmanni* Pantocs.). *Glasnik Zemaljskog Muzeja Bosne i Hercegovine Sarajevo, V,* 105-108 (in Bosnian).

Slovak, M., Vít, P., Urfus, T., Suda, J. (2009). Complex pattern of genome size variation in a polymorphic member of the Asteraceae. *Journal of Biogeography, 36,* 372-384.

Souza, R.P., Válio, I.F.M. (2003). Leaf optical properties as affected by shade in saplings of six tropical tree species differing in successional status. *Brazilian Journal of Plant Physiology, 15,* 49-54.

Tutin, T.G. (1996). *Symphyandra* AD. In Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M.I., Webb, D.A. (Ed.), *Flora Europaea*. Cambridge University Press, 4-93.

Summary/Sažetak

U ovom istraživanju ispitivan je sadržaj fotosintetskih pigmenata, ukupnih antocijanina i veličina genoma u šest prirodnih populacija endemične vrste *Symphyandra hofmannii* Pant. (Bosanska zvončika ili Hofmanova zvončika) iz Bosne i Hercegovine. Izvršena je intrapopulacijska i interpopulacijska analiza vrste *Symphyandra hofmannii* koja je rasla u različitim stanišnim uvjetima. Interpopulacijska analiza individua koje su se razvijale u različitim ekološkim uvjetima pokazala je statistički značajne razlike za sve ispitivane parametre (hlorofil *a* i *b*, ukupni hlorofili, odnos hlorofila *a*/*b*, antocijanina, karotenoida i količini DNK). Uočene su i statistički značajne razlike između individua iz iste populacije ali različitih habitata u sadržaju hlorofila b, odnosu hlorofila *a*/*b* i antocijana. Interpopulacijska varijabilnost u sadržaju pigmenata nije se mogla pripisati svjetlosti kao jedinom diferencijalnim ekološkom parametru. Ova varijabilnost je bila uslovljena kombinacijom sredinskih faktora kao što su geološka podloga, nadmorska visina ili antropogeni faktori. Veličina genoma signifikantno je odstupala samo kod jedine serpentniske populacije (Papratnica).

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2015

44

79-82

Print ISSN: 0367-4444
Online ISSN: 2232-7266

UDC: _____

Technical paper

Investigation of potentially contaminated areas in the Federation of Bosnia and Herzegovina with depleted uranium

Nuhanović, M.[a]*, Vidic, A.[b], Ilić, Z.[b], Kaldžija, N.[a], Pušina, N.[a]

[a] *University of Sarajevo, Faculty of Science, Department of Chemistry, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina*

[b] *Institute for Pablic Health of FBiH, M.Tita 9, 71000 Sarajevo, Bosnia and Herzegovina*

Article info
Received: 31/10/2014
Accepted: 04/06/2015

Keywords:
ammunition depleted uranium
contamination
alpha-spectrometry
determination of uranium

***Corresponding author:**
E-mail: mirza.nuhanovic@yahoo.com
Tel: +387/33/279-865

Abstract: During the war in Bosnia, depleted uranium was used on several - locations in Bosnia and Herzegovina, including the area of Hadzici. Estimated amount of used ammunition is close to three tons. Only a fraction of depleted uranium penetrator, detected in the surface ground layer was removed. A certain number of ground, moss and subterranean water samples have been collected in december 2013, for the purpose of evaluation of two decade long contamination from depleted uranium ammo usage. The colected samples were subjected to radiochemical separation and alpha-spectrometric analysis. The results of the examination showed that the uranium was present in the amount of 0.6 to 1.8 μg/kg in the ground samples, 0.2 to 7.0 μg/kg in the moss samples and 0.36 to 1.04 μg/L in the subterranean water. The activity ratio of $^{234}U/^{238}U$ in three moss samples, as well as one ground sample, showed the presence of depleted uranium. Analyzed water samples indicated a natural relation of uranium isotopes. Tests shows that the presence of depleted uranium deserves detailed examination of radioactivity, radioecology assessment and evaluation of population exposure.

INTRODUCTION

During the last war in Bosnia, depleted uranium was used on several locations in Bosnia and Herzegovina, including the area of Hadzici. Estimated amount of used ammunition is close to three tons. The largest proportion of ammunition, whole penetrator or fragment, is buried deeper in the ground, where, depending on the chemical and other corrosive conditions, migrates into the environment and is a potential source of groundwater contamination. Only a fraction of depleted uranium penetrator, detected in the surface ground layer was removed. According to this, a certain number of surface soil, moss and groundwater samples were collected in December 2013. The purpose of that was to evaluate the contamination of this specific area, two decades following the use of depleted uranium ammo. Groundwater and surface water contamination has been identified as a major issue of concern for long-term testing. The size and chemical characteristics of the particles, as well as the local geochemical and hydrogeological characteristics exert a decisive influence on the migration of uranium. Also, the different chemical forms of radionuclides, present in the soil are available for plants and its absorption. Considering that, the tests are performed twenty years after the use of depleted uranium in the area tested, contamination of plants could be carried through radionuclide transfer from soil to plants via the root system. Bettencourt et al., (1988), have found that various factors, such as soil characteristics, climate, plants and their parts, the physical and chemical form of radionuclides and the effect of competing organism (organism that compete with plants for food) may affect the value of transfer factors.

The aim of this research is to determine uranium isotopes content in the collected samples of the ground,

moss and water, as well as to investigate the total alpha and beta activities of the selected samples. The collected samples were subjected to separation and alpha radiochemical analysis. Tests show that the presence of depleted uranium deserves detailed examination of radioactivity, radioecology assessment and evaluation of population exposure.

EXPERIMENTAL

Ground samples were collected from nine different locations, water samples from three, and moss samples from five locations. The samples of the ground and moss were cleaned from mechanical impurities (stones, solid soil particles from moss) and then dried at room temperature, after which they were brought to the state of constant mass by application of the intermittent dry-heat procedure. Tracer (^{232}U) and mixture of acids (Aqua regia), were added to a specific amount of each sample, and together dried out. The dry residue was dissolved in concentrated HCl. The soultions were released through the ion exchange resin (Dowex 1x8), Cl-form, 100-200. Thorium was removed by releasing 8 mol/L HCl through ion exchange column, after which uranium was eluated by 25 mL 0.5 mol/L HCl. The samples were prepared for measurement by microprecipitacion with NdF_3, according to the HASL-300 procedure (EML, 28th Ed. 1997). The Nd-carrier, $TiCl_3$ as uranium reducer and concentrated HF was added to the solution containing uranium. Following that, the samples were kept in ice and then filtered through a membrane 0.22 μm filter paper. Filter paper was fixed to the planchete and then dried by UV lamp for fifteen minutes. The measurement was performed by alphaspectrometer (Alpha Analyst Canberra) equipped with silicium detectors (PIPS) with 450 mm^2 of active surface area. The measurement time was 172800 seconds and chemical yield was 20-98%. The lower limit of detection was 0.18–0.25 mBq/L for ^{238}U, 0.12–0.17 mBq/L for ^{235}U, & 0.20–0.28 mBq/L for ^{234}U.

The collected water samples were taken to laboratory without acidification, were then filtered through 0.45 μm filter paper, and dry residue determined (105°C). Approximatelly 2 L of water were taken for the analysis from each sample. A uranium tracer ^{232}U, approximately 15 mBq, was added to measured aliquotes of water samples in order to calculate chemical yield. Uranium was coprecipicated with $Ca_3(PO_4)_2$, according to the Eichrom procedure (Eichrom Technologies Inc. Ver 1.7.2011). After centrifuge, deposit $Ca_3(PO_4)_2$ was dissolved in 5 mL 8 mol/L HCl.

The further procedure with regard to water samples is identical to that of ground and moss as described above.

An isotope analysis of uranium was performed in the examined samples, as well as a total alpha-beta activity for moss and ground samples. The data of the analyzed samples of moss, ground and water are presented in the following tables and diagrams.

Table 1. Moss samples impulse responses measured by Alpha-spectrometry

Moss samples	N (^{238}U) Imp	N (^{235}U) Imp	N (^{234}U) Imp	N (^{232}U) Imp
1	441	39	183	495
2	534	16	386	546
3	100	12	106	286
4	1682	40	339	286
5	4350	71	671	328

The table clearly shows, based on the value of pulses for the ^{235}U, that the analyzed samples of moss contain depleted uranium. Similar results were obtained for the analyzed samples of soil and water.

Table 2. Results of uranium radioisotopes activity obtained through Alpha-spectrometric analysis of moss samples

Moss samples	A(^{238}U) (Bq/kg)	A(^{234}U) (Bq/kg)	Mass(U) (mg/kg)	Activity ratio ^{234}U/^{238}U	Chemical yield
1	5.17	2.03	0.4	0.39	77%
2	8.51	6.02	0.7	0.71	73%
3	2.74	2.71	0.2	0.99	39%
4	45.31	8.84	3.6	0.19	34%
5	86.57	13.13	7.0	0.15	36%

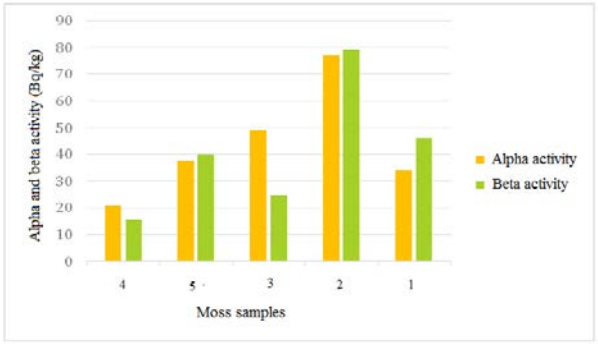


Diagram 1. Gross alpha and beta activity of analyzed moss samples

Determination of gross alpha and beta activity of the analyzed samples was performed in order to verify the presence of radioactive elements in analyzed samples. Results indicated the presence of alpha and beta emitters in moss and ground analyzed samples (Diagrams 1&2).

Table 3. Results of uranium radioisotopes activity obtained through Alpha-spectrometric analysis of ground samples

Ground samples	A(^{238}U) (Bq/kg)	A(^{234}U) (Bq/kg)	Mass(U) (mg/kg)	Activity ratio ^{234}U/^{238}U	Chemical yield
1	18.1	17.3	1.5	0.95	30%
2	11.3	11.4	0.9	1.01	56%
3	7.7	8.3	0.6	1.07	37%
4	19.6	20.7	1.6	1.06	21%
5	13.4	15.8	1.1	0.69	20%
6	22.3	18.8	1.8	1.10	27%
7	16.1	11.1	1.3	1.17	25%

The pulses value for two analyzed samples of the ground is at the lower limit of detection, so for them there was no need to count the active concentration of uranium isotopes.

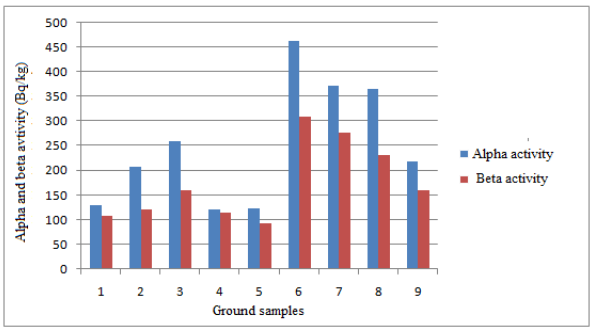


Diagram 2. Gross alpha and beta activity of analyzed ground samples

Table 4. Results of uranium radioisotopes activity obtained through Alpha spectrometric analysis of water samples

Water samples	A(^{238}U) (Bq/L)	A(^{234}U) (Bq/L)	Mass(U) (μg/L)	Activity ratio ^{234}U/^{238}U	Chemical yield
1	0.0045	0.0049	0.36	1.09	72%
2	0.0129	0.0175	1.04	1.35	94%
3	0.0112	0.0160	0.90	1.43	98%

The results of analysis show that the uranium content in the ground samples range from 0.6 to 1.8 mg/kg, in the moss samples from 0.2 to 7.0 mg/kg, and in the water samples, from 0.36 to 1.04 μg/L.

The analysed samples of water show presence of the natural ratio of uranium isotopes. Radioactivity of the examined water samples is relativelly low. The results for the investigated waters are considerably lower than World Health Organisation (WHO) drinking water guideline limit value of 30 μg/L for total uranium (WHO, 2011). The data obtained through analysis of the water samples are close to the date obtained in years 2003 (UNEP, 2003), (Jia G, et.al., 2006) and (Vidic A. et al., 2013). The same can be said for most of the surface ground samples, as well as for two of the moss samples. However, since uranium concentration can increase significantly within relativelly short period of time in case of contamination of water, a monitoring of uranium concentration in underground waters is necessary.

The ratio of ^{234}U/^{238}U activity in four samles of moss, as well as in one sample of ground, show presence of depleted uranium. Similar results were obtained through analysis of vegetation and ground (several samples) performed by UNEP (UNEP, 2003). The obtained data shows that the contamination by depleted uranium in the surface layer has been present 20 years after the use of depleted uranium in this area.

CONCLUSIONS

Radioactivity of water samples is relativelly low. Concentration of activity of uranium isotopes is low and ranges from 0.36 to 1.04 μg/L of uranium mass concentration.

The ratio of ^{234}U/^{238}U activity in four samles of moss, as well as in one sample of ground, show presence of depleted uranium, which indicates the need of detailed survey (of greater number of samples of ground, water and vegetation), in order to obtain more accurate data on contamination of the examined area.

The analysis shows that the presence of depleted uranium points to the need to conduct a detailed analysis of radioactivity, radio-environmental evaluation, and evaluation of exposure of the population.

REFERENCES

Bettencourt, A.O., Teixeira, M.M.G.R., Elias, M.D.T., Faisca, M.C. (1988). Soil to plant transfer of Radium-226, *Journal of Environmental Radioactivity* 6, 49-60.

Eichrom Technologies Inc. Analytical procedures Uranium and Thorium in Water-ACW03. Ver 1.7. 2001.

Environmental Measurements Laboratories (EML). (1997) Microprecipitation source preparation for alpha spectrometry. EML Procedures Manual-HASL 300.28th Ed.

Jia G., Belli M., Sansone U., Rosamilia S., Gaudino S. (2006). Concentration and characteristics of depleted uranium in biological and water samples collected in Bosnia and Herzegovina. *Journal of Environmental Radioactivity* 89:172-187.

UNEP.(2003). Depleted Uranium in Bosnia and Herzegovina, Post-Conflict Environmental Assessment.

Vidic A, Rašidagić A, Ilić Z, Nuhanović M . (2013). Recent Determinations of Uranium and Gross Alpha and Betain Groud water Samples of Hadzici Area, Proceedings of the 9th Symposium of the CROATIAN RADIATION PROTECTION ASSOCIATION, Zagreb.

World Health Organization. (2011). Guidelines for drinking-water quality, 4th Ed. WHO.

Summary/Sažetak

Tokom rata u BiH, osiromašeni uran je upotrebljen na nekoliko lokacija u BiH, uključujući područje Hadžića. Procjenjena količina upotrebljene municije iznosi približno 3 tone. Samo dio penetratora od osiromašenog urana, detektovan u površinskom sloju tla, je uklonjen. U decembru 2013. godine je prikupljen određen broj uzoraka tla, mahovina i podzemnih voda, u cilju procjene kontaminacije dvije decenije nakon upotrebe municije od osiromašenog urana. Prikupljeni uzorci su podvrgnuti radiohemijskoj separaciji i alfa spektrometrijskoj analizi. Rezultati ispitivanja pokazuju da sadržaj urana u uzorcima tla iznosi od 0.6 do 1.8 mg/kg, u uzorcima mahovina od 0.2 do 7.0 mg/kg, i uzorcima vode od 0.36 do 1.04 µg/L. Odnos aktivnosti $^{234}U/^{238}U$ u tri uzorka mahovina, kao i jednom uzorku tla ukazuje na prisustvo osiromašenog urana. Analizirani uzorci vode pokazuju prirodan odnos uranovih izotopa. Ispitivanja pokazuju da prisustvo osiromašenog urana zavređuje detaljno ispitivanje radioaktivnosti, radioekološku procjenu i procjenu izloženosti stanovništva.

DKT KS
DRUŠTVO KEMIČARA I TEHNOLOGA KANTONA SARAJEVO
BOSNA I HERCEGOVINA
Zmaja od Bosne 35, 71000 Sarajevo, Bosna i Hercegovina
Tel. +387 33 279 911
Web: www.dktks.ba, E-mail: dktks.sekretar@gmail.com

Sarajevo, 11.12.2014.

2. Redovna Skupština Društva kemičara i tehnologa Kantona Sarajevo

će se održati u ponedjeljak 15. decembra 2014. godine u

16:15 sati, u hemijskom amfiteatru (amfiteatar Mladen Deželić),

Odsjek za hemiju, PMF

Prijedlog dnevnog reda:

1. Izbor zapisničara (2), i ovjerivača zapisnika (2).
2. Izvještaj Upravnog odbora o radu i stanju Društva i njegovih sekcija
3. Usvajanje finansijskog Izvještaja za proteklu godinu
4. Utvrđivanje prijedloga proračuna za narednu godinu (prijedlog UO)
5. Utvrđivanje prijedloga aktivnosti za 2015. g
6. Najava i pripreme za Izbornu skupštinu
7. Razno

Predsjednik Upravnog Odbora
Harun Kurtagić

DKT KS
DRUŠTVO KEMIČARA I TEHNOLOGA KANTONA SARAJEVO
BOSNA I HERCEGOVINA
Zmaja od Bosne 35, 71000 Sarajevo, Bosna i Hercegovina
Tel. +387 33 279 911
Web: www.dktks.ba, E-mail: dktks.sekretar@gmail.com

DRUŠTVO KEMIČARA I TEHNOLOGA
KANTONA SARAJEVO

Sarajevo, 15.12.2014.

ZAPISNIK

2. Redovne Skupštine Društva kemičara i tehnologa Kantona Sarajevo

koja je održana u ponedjeljak 15. decembra 2014. godine u

16:15 sati, u hemijskom amfiteatru (amfiteatar Mladen Deželić),

Odsjek za hemiju, PMF

Prisutni:

1. Mustafa Memić	15. Lejla Klepo
2. Fehim Korać	16. Sabina Gojak-Salimović
3. Tarik Fetahagić	17. Harun Kurtagić
4. Jelena Ostojić	18. Anes Krečo
5. Dalibor Karačić	19. Aida Konjević
6. Sabina Žero	20. Emira Hodžić
7. Mirza Nuhanović	21. Emina Ramić
8. Sabina Begić-Hairlahović	22. Alema Dedić
9. Omer Mahmutović	23. Almir Olovčić
10. Mevludin Hajdar	24. Tidža Muhić-Šarac
11. Melisa Tvrtković	25. Nurudin Avdić
12. Enis Mašnić	26. Šaćira Mandal
13. Nevzeta Ljubijankić	27.Hajrudin Hajdar
14. Danijela Vidic	28. Dajana Lekić

Hajrudin Hajdar pozdravio je prisutne i pročitao je predloženi dnevni red koji glasi:

Prijedlog dnevnog reda:

1. Izbor zapisničara i ovjerivača zapisnika (2).
2. Izvještaj Upravnog odbora o radu i stanju Društva i njegovih sekcija
3. Usvajanje finansijskog Izvještaja za proteklu godinu
4. Utvrđivanje prijedloga proračuna za narednu godinu (prijedlog UO)

Zmaja od Bosne 35, 71000 Sarajevo, Bosna i Hercegovina
Tel. +387 33 279 911
Web: www.dktks.ba, E-mail: dktks.sekretar@gmail.com

5. Utvrđivanje prijedloga aktivnosti za 2015. g
6. Najava i pripreme za Izbornu skupštinu
7. Razno

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

1. Izbor zapisničara i ovjerivača zapisnika (2).

Za zapisničara je predložena Ostojić Jelena, a za ovjerivače zapisnika Sabina Žero i Lejla Klepo.

Jednoglasno usvojeni prijedlozi.

2. Izvještaj Upravnog odbora o radu i stanju Društva i njegovih sekcija

Harun Kurtagić kao predsjednik Upravnog odbora je pripremio Izvještaj o radu Društva za 2014.godinu. Izvještaj u printanoj formi će biti priložen uz Zapisnik. Imenovani je ukratko obrazložio koliko je UO održao sastanaka, te šta je sve urađeno i pokrenuto od strane UO.
Ono što je uraženo u protekloj godini može se kroz najbitnije cjelini sažeti na slijedeće:

Obnovljena registracija
Revidiran Statut
Obnovljeno članstvo
Pokrenuta web stranica
Održan uspješan međunarodni kongres hemičara i tehnologa BiH
Stanje kase popravljeno

Kao najveći problem u radu Društva Kurtagić navodi nezadovoljavajući odziv pojedinih članova, nezainteresovanost tijela Društva, te nedostatak Pravilnika o radu.

Harun Kurtagić na kraju svog izlaganja zahvalio se članovima koji su prisutni, nada se da je dijelom uspio ostvariti napredak Društva. On lično naveo je i nije toliko zadovoljan, te se nada da ukoliko dođe neko drugi na tu funkciju uspjeti uraditi više.

Riječ je preuzeo Hajrudin Hajdar, koji se zahvalio predsjedniku Društva na izlaganju. Zahvalio se također ljudima koji su dali najveći doprinos organizaciji Kongres hemičara. Harudin Hajdar je otvorio diskusiju. S obzirom da nije bilo prjavljenih diskutanata, tačka je stavljena na glasanje.

Izvještaj o radu Društva u prethodnoj godini jednoglasno je usvojen.

Zmaja od Bosne 35, 71000 Sarajevo, Bosna i Hercegovina
Tel. +387 33 279 911
Web: www.dktks.ba, E-mail: dktks.sekretar@gmail.com

3. Usvajanje finansijskog Izvještaja za proteklu godinu

Finansijski Izvještaj je sastavila blagajnik Sabina Žero. Nadzorni odbor nije potpisao Izvještaj, ali su ga pregledali i dali usmenu saglasnost. Predat je godišnji obračun za 2013.godinu.

Jednoglasno usvojen finansijki Izvještaj.

4. Utvrđivanje prijedloga proračuna za narednu godinu (prijedlog UO)

Proračun za 2015.g nije napravljen jer nisu ostvareni svi ciljevi naveo je Harun Kurtagić.

5. Utvrđivanje prijedloga aktivnosti za 2015. g

Harun Kurtagić naveo je ciljeve za narednu godinu, a to su:

-Izrada pravilnika o radu
- Formiranje odbora i komisija
- Finansijska opstojnost Društva
-Prikupljanje članarine

Hajrudin Hajdar predložio je da se i ostali članovi uključe u izradu prijedloga proračuna za narednu godinu, kao i plana aktivnosti. Prijedloge trebaju poslati sekretaru i blagajniku.

6. Najava i pripreme za Izbornu skupštinu

Hajrudin Hajdar je naveo da se Izborna Skupština treba održati najkasnije do kraja februara, jer mandati za trenutne članove tijela Društva ističu 11. marta. Predložena Komisija za izbor je:

1. Hajrudin Hajdar
2. Mustafa Memić
3. Šaćira Mandal

Komisija se treba sastati do kraja januara da sastavi prijedloge za nove članove upravnih tijela. Memić Mustafa treba koordinirati i sazvati sastanak Komisije.

Članovi Društva složili su se sa navedenim prijedlogom.

DKT KS
DRUŠTVO KEMIČARA I TEHNOLOGA KANTONA SARAJEVO
BOSNA I HERCEGOVINA
Zmaja od Bosne 35, 71000 Sarajevo, Bosna i Hercegovina
Tel. +387 33 279 911
Web: www.dktks.ba, E-mail: dktks.sekretar@gmail.com

7. Razno

a) Šaćira Mandal postavila pitanje vezano za Pravilnik o radu, da li taj pravilnik treba da izradi trenutni UO ili novi. Prijedlog je da trenutni UO napravi nacrt pravilnika.
b) Mustafa Memić se javio za diskusiju i iznio njegovo mišljenje o stanju Društva.

Nakon izlaganja Mustafe Memića nije bilo više prijavljenih za diskusiju, te je ovim izlaganjem završena 2. Skupština Društva.

Skupština završena u 17:30.

Sarajevo, 15.12.2014. godine

Zapisničar:

_____
Jelena Ostojić

Ovjerivači zapisnika:

_____
Lejla Klepo

_____
Sabina Žero

Predsjednik Skupštine:

_____
Hajrudin Hajdar

Društvo kemičara i tehnologa Kantona Sarajevo

Sarajevo, 14.02.2015.

Z A P I S N I K

SA 2. IZBORNE SKUPŠTINE DRUŠTVA KEMIČARA I TEHNOLOGA KANTONA SARAJEVO

održane u subotu, 14. februara 2015. godine sa početkom u 11:00 sati na Prirodno-matematičkom fakultetu Sarajevo.

Gospodin Hajrudin Hajdar, predsjednik Skupštine Društva pozdravio je sve prisutne i otvorio Izbornu Skupštinu Društva. Konstatovao je da **Skupštini prisustvuje 53 člana Društva i da skupština ima kvorum te da može donositi odgovarajuće odluke.**

Gospodin Hajrudin Hajdar je pročitao dnevni red današnje Skupštine koji je ranije dostavljen svim članovima Društva.

Predložen je sljedeći dnevni red:P

1. Izbor radnog Predsjedništva
2. Izbor zapisničara
3. Izbor 2 ovjerivača Zapisnika.
4. Usvajanje Zapisnika sa 2. Redovne Skupštine
5. Izbor članova Skupštine Društva
6. Izvještaj o radu Skupštine u prethodnoj godini
7. Izbor članova Upravnog odbora, Nadzornog odbora i Suda časti
8. Razno

Dnevni red je bez izmjena jednoglasno usvojen.

Nakon što je prihvaćen dnevni red, gospodin Hajdar održao je kratki govor o Društvu uopšte i prethodno obavljenim aktivnostima koje se odnose na rad Društva. Obavijestio je prisutne, također, da je potrebno izabrati novo rukovodstvo Društva, s obzirom da trenutnom rukovodstvu Društva ističe dvogodišnji mandat. Naglasio je da se izborom novih tijela Društva, na ovoj Skupštini, dosadašnji članovi upravnih tijela Društva razrješavaju dužnosti iz razloga isteka perioda na koji su imenovani.

Gospodin Hajdar je potom predložio da skupština počne raditi po prihvaćenom dnevnom redu.

AD 1. Izbor radnog predsjedništva

Predložio je da se po tački 1. izabere radno predsjedništvo, za šta su predloženi Harun Kurtagić, Sabina Gojak-Salimović i Hajrudin Hajdar.

Prijedlog je jednoglasno prihvaćen.

AD 2. Izbor zapisničara

Gosp. Hajrudin Hajdar je nastavio dalje voditi sjednicu, te predložio za zapisničara Ostojić Jelenu.

Prijedlog gosp. Hajdara je jednoglasno usvojen.

AD 3. Izbor 2 ovjerivača zapisnika

Za ovjeru zapisnika predložena su dva člana Društva i to, Sabina Žero i Lejla Klepo.

Prijedlog gosp. Hajdara je jednoglasno usvojen.

AD 4. Usvajanje Zapisnika sa 2. Redovne Skupštine

Hajrudin Hajdar je obavjestio prisutne da su u pozivu na Skupštinu svi članovi Društva kao prilog dobili Zapisnik sa 2. Redovne Skupštine. Pitao je prisutne da li ima primjedbi na Zapisnik. Kako nije bilo primjedbi na zapisnik isti je stavljen na usvajanje.

Zapisnik je jednoglasno usvojen.

AD 5. Izbor članova Skupštine

Prema Statutu Društva prijedloge za članove upravnih tijela Društva predlažu članovi Društva dostavljajući pismene prijedloge, najkasnije 5 dana prije održavanja izborne Skupštine.
Na osnovu prispjelih prijedloga predloženi su sljedeći članovi upravnih tijela društva iz reda članova Društva (u daljem tekstu dati su prijedlozi za pojedina tijela Društva):

Za Skupštinu Društva predloženi su:

Predsjednik Skupštine: **Mustafa Memić**
Zamjenik predsjednika Skupštine: **Šaćira Mandal**
Glavni i odgovorni urednik društvenih glasila: **Fehim Korać**

Glasalo se javnim glasanjem za svaki pojedinačni prijedlog.

Predloženi kandidati su jednoglasno izabrani.

Nakon izbora novog predsjednika Skupštine Društva, dosadašnji predsjednik Hajrudin Hajdar je dalje vođenje Skupštine prepustio novoizabranom predsjedniku Skupštine Mustafi Memiću. Gospodin Memić se zahvalio članovima Društva na ukazanom poverenju te održao kratki govor. Potom je nastavio voditi sjednicu po dnevnom redu i prema tački 6. dnevnog reda pozvao dosadašnjeg predsjednika Skpštine Hajrudina Hajdara da podnese Izvještaj o radu prethodne Skupštine.

AD 6. Izvještaj o radu prethodne Skupštine

Hajrudin Hajdar iznio je kratki Izvještaj o radu u toku prethodne 2 godine. Izvještaj u pisanoj formi biće priložen uz Zapisnik.

Prešlo se na sedmu tačku dnevnog reda.

AD 7. Izbor članova Upravnog odbora, Nadzornog odbora i Suda časti

a) Za članove **Upravnog odbora** predloženi su sljedeći kandidati:

Predsjednik: **Edhem Mulaosmanović**

Potpresjednik:
1. **Omer Mahmutović**
2. **Almir Olovčić**
3. **Josip Jurković**

Sekretar : **Jelena Ostojić**
Blagajnik: **Sabina Žero**
Urednik glasila: **Safija Herenda**

Za još dva člana UO predloženi su sljedeći kandidati:

1. Indira Kozica
2. Harun Kurtagić
3. Hurija Džudžević Čančar
4. Dragan Krešić
5. Amela Hrbat
6. Semira Galijašević
7. Zlatan Rimpapa
8. Emina Ramić
9. Dajana Lekić
10. Enis Mašnić
11. Mevludin Hajdar

Na Skupštini je odlučeno da se za članove upravnih tijela gdje je predložen jedan kandidat glasa javnim izjašnjavanjem podizanjem ruku članova Društva a za članove upravnih tijela gdje je predloženo više kandidata glasa tajnim izjašnjavanjem članova Društva za šta su pripremljeni odgovarajući glasački listići. Za prebrojavanje glasova Skupština je predložila Mandal Šaćiru i Ostojić Jelenu.

Javnim glasanjem su jednoglasno izabrani sljedeći članovi Upravnog odbora društva:

Predsjednik: **Edhem Mulaosmanović**
Sekretar : **Jelena Ostojić**
Blagajnik: **Sabina Žero**
Urednik glasila: **Safija Herenda**

Za potpredsjednika i preostala dva člana UO bilo je više predloženih kandidata, te se glasalo preko izbornih listića.

Za potpredsjednika Upravnog odbora je većinom glasova članova Društva (25 osvojenih glasova) izabran **Almir Olovčić.**

Još dva člana UO izabrana su većinom glasova članova Društva i to:

1. **Emina Ramić** sa 18 osvojenih glasova i
2. **Dragan Krešić** sa 17 osvojenih glasova

Tako će u naredne dvije godine Upravni odbor Društva činiti sljedeći članovi:

1. Predsjednik: **Edhem Mulaosmanović**
2. Potpresjednik: **Almir Olovčić**
3. Sekretar : **Jelena Ostojić**
4. Blagajnik: **Sabina Žero**
5. Urednik glasila: **Safija Herenda**
6. Član: **Emina Ramić**
7. Član: **Dragan Krešić**

Nakon izbora članova Upravnog odbora, prešlo se na izbor tri članoa Nadzornog odbora i pet članova Suda časti.

b) Za članove **Nadzornog odbora** predloženi su sljedeći kandidati:

Predsjednik: **Aida Šapčanin**
Član: **Hajrudin Hajdar**
Član: **Jozo Ćorić**

Predloženi članovi Nadzornog odbora su jednoglasno podržani.

c) Za članove **Suda časti** predloženi su sljedeći kandidati:

Član: **Faiza Muštović-Biščević**
Član:**Ismet Tahirović**
Član: **Borivoj Galić**
Član: **Nurudin Avdić**
Član: **Mirza Nuhanović**

Predloženi članovi Suda časti su jednoglasno podržani.

AD 8. Razno

Harun Kurtagić kao bivši predsjednik UO i ujedno i predsjednik Društva zatražio je da se obrati članovima Društva. Obrazložio je ukratko probleme sa kojima se susretao UO u toku svog rada, i iskoristio priliku da čestita novoizabranim članovima upravnih tijela i poželi im sreću u naredne dvije godine.

Mustafa Memić je upoznao članove ukratko o održanom Kongresu hemičara i tehnologa BiH koji je održan u oktobru 2014., zatim je podsjetio članove Društva o zadacima i obavezama Društva i njegovih članova, naglašavajući da je Društvo izraslo u Društvo sa respektaktivnim brojem članova i da bi trebalo raditi na osnivanju određenih sekcije Društva kako je predviđeno Statutom Društva.

Novoizabrani predsjednik Upravnog Odbora, predsjednik Društva Edhem Mulaosmanović se predstavio i kratko obratio članovima Društva.

Sjednica završena u 12:15h.

Zapisničar

_____

Jelena Ostojić

Predsjednik Skupštine

_____

Mustafa Memić

Ovjerivači zapisnika:

_____

Sabina Žero

_____

Lejla Klepo

DKT KS
DRUŠTVO KEMIČARA I TEHNOLOGA
KANTONA SARAJEVO
BOSNA I HERCEGOVINA
Zmaja od Bosne 35, 71000 Sarajevo, Bosna i Hercegovina
Tel. +387 33 279 911
Web: www.dktks.ba, E-mail: dktks.sekretar@gmail.com

Na osnovu člana 18. Zakona o udruženjima i fondacijama ("Službene novine Federacije BiH", broj 45/02), i člana 19. Statuta Udruženja "Društvo kemičara i tehnologa Kantona Sarajevo" Skupština Društva na sjednici održanoj dana 14.02.2015. godine, donijela je

ODLUKU O RAZRJEŠENJU PREDSJEDNIKA I ČLANOVA

Upravnog Odbora
Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

Član 1.

Razrješavaju se dužnosti članovi Upravnog odbora udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“:

1. Harun Kurtagić, predsjednik
2. Aida Šapčanin, podpredsjednik
3. Jelena Ostojić, sekretar
4. Sabina Žero, blagajnik
5. Edina Hodžić, glavni urednik
6. Tarik Fetahagić, član
7. Amela Hrbat, član

Član 2.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-01-1/15

14. februar 2015.g

Sarajevo

Predsjednik Skupštine

Memić Mustafa

DKT KS
DRUŠTVO KEMIČARA I TEHNOLOGA
KANTONA SARAJEVO
BOSNA I HERCEGOVINA
Zmaja od Bosne 35, 71000 Sarajevo, Bosna i Hercegovina
Tel. +387 33 279 911
Web: www.dktks.ba, E-mail: dktks.sekretar@gmail.com

Na osnovu člana 18. Zakona o udruženjima i fondacijama ("Službene novine Federacije BiH", broj 45/02), i člana 19. Statuta Udruženja "Društvo kemičara i tehnologa Kantona Sarajevo" Skupština Društva na sjednici održanoj dana 14.02.2015. godine, donijela je

ODLUKU O RAZRJEŠENJU ČLANOVA

Nadzornog Odbora
Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

Član 1.

Razrješavaju se dužnosti članovi Nadzornog odbora udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“ :

1. Faiza Muštović Bišćević, predsjednik
2. Sead Hrustanović, član
3. Ismet Tahirović, član

Član 2.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-01-2/15

14. februar 2015.g

Sarajevo

Predsjednik Skupštine

Memić Mustafa

DKT KS
DRUŠTVO KEMIČARA I TEHNOLOGA
KANTONA SARAJEVO
BOSNA I HERCEGOVINA
Zmaja od Bosne 35, 71000 Sarajevo, Bosna i Hercegovina
Tel. +387 33 279 911
Web: www.dktks.ba, E-mail: dktks.sekretar@gmail.com

Na osnovu člana 18. Zakona o udruženjima i fondacijama ("Službene novine Federacije BiH", broj 45/02), i člana 19. Statuta Udruženja "Društvo kemičara i tehnologa Kantona Sarajevo" Skupština Društva na sjednici održanoj dana 14.02.2015. godine, donijela je

ODLUKU O RAZRJEŠENJU ČLANOVA

Suda časti
Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

Član 1.

Razrješavaju se dužnosti članovi Suda časti udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“:

1. Emin Sofić, član
2. Zlatan Rimpapa, član
3. Borivoj Galić, član

Član 2.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-01-3/15

14. februar 2015.g

Sarajevo

Predsjednik Skupštine

Memić Mustafa

DKT KS
DRUŠTVO KEMIČARA I TEHNOLOGA
KANTONA SARAJEVO
BOSNA I HERCEGOVINA
Zmaja od Bosne 35, 71000 Sarajevo, Bosna i Hercegovina
Tel. +387 33 279 911
Web: www.dktks.ba, E-mail: dktks.sekretar@gmail.com

Na osnovu člana 18. Zakona o udruženjima i fondacijama ("Službene novine Federacije BiH", broj 45/02), i člana 19. Statuta Udruženja "Društvo kemičara i tehnologa Kantona Sarajevo" Skupština Društva na sjednici održanoj dana 14.02.2015. godine, donijela je

ODLUKU O RAZRJEŠENJU PREDSJEDNIKA, POTPREDSJEDNIKA I GLAVNOG UREDNIKA DRUŠTVENIH GLASILA Skupštine Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

Član 1.

Razrješavaju se dužnosti predsjednik, potpredsjednik i glavni urednik društvenih glasila Skupštine udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“:

1. Hajrudin Hajdar, predsjednik
2. Dalibor Karačić, podpredsjednik
3. Fehim Korać, glavni urednik društvenih glasila

Član 2.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-01-4/15

14. februar 2015.g

Sarajevo

Predsjednik Skupštine

Memić Mustafa

DKT KS
DRUŠTVO KEMIČARA I TEHNOLOGA
KANTONA SARAJEVO
BOSNA I HERCEGOVINA
Zmaja od Bosne 35, 71000 Sarajevo, Bosna i Hercegovina
Tel. +387 33 279 911
Web: www.dktks.ba, E-mail: dktks.sekretar@gmail.com

Na osnovu člana 18. Zakona o udruženjima i fondacijama ("Službene novine Federacije BiH", broj 45/02), i člana 19. Statuta Udruženja "Društvo kemičara i tehnologa Kantona Sarajevo" Skupština Društva na sjednici održanoj dana 14.02.2015. godine, donijela je

ODLUKU O IMENOVANJU ČLANOVA

Skupštine Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

Član 1.

U Skupštinu udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“ imenuju se :

1. Mustafa Memić, predsjednik
2. Šaćira Mandal, podpredsjednik
3. Fehim Korać, glavni urednik društvenih glasila

Član 2.

Izabrani članovi Skupštine Udruženja su postali kandidati sa najvećim brojem glasova. Mandat članova Skupštine traje 2 godine.

Član 3.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-02/15

14. februar 2015.g

Sarajevo

Predsjednik Skupštine

Memić Mustafa

DKT KS
DRUŠTVO KEMIČARA I TEHNOLOGA
KANTONA SARAJEVO
BOSNA I HERCEGOVINA
Zmaja od Bosne 35, 71000 Sarajevo, Bosna i Hercegovina
Tel. +387 33 279 911
Web: www.dktks.ba, E-mail: dktks.sekretar@gmail.com

Na osnovu člana 18. Zakona o udruženjima i fondacijama ("Službene novine Federacije BiH", broj 45/02), i člana 19. Statuta Udruženja "Društvo kemičara i tehnologa Kantona Sarajevo" Skupština Društva na sjednici održanoj dana 14.02.2015. godine, donijela je

ODLUKU O IMENOVANJU PREDSJEDNIKA I ČLANOVA

Upravnog Odbora
Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

Član 1.

U Upravni odbor udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“ imenuju se :

1. Edhem Mulaosmanović, predsjednik
2. Almir Olovčić, potpredsjednik
3. Jelena Ostojić, sekretar
4. Sabina Žero, blagajnik
5. Safija Herenda, glavni urednik
6. Dragan Krešić, član
7. Emina Ramić, član

Član 2.

Izabrani članovi Upravnog odbora Udruženja su postali kandidati sa najvećim brojem glasova. Mandat članova Upravnog odbora traje 2 godine.

Član 3.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-03/15

14. februar 2015.g

Sarajevo

Predsjednik Skupštine

Memić Mustafa

DKT KS
DRUŠTVO KEMIČARA I TEHNOLOGA
KANTONA SARAJEVO
BOSNA I HERCEGOVINA
Zmaja od Bosne 35, 71000 Sarajevo, Bosna i Hercegovina
Tel. +387 33 279 911
Web: www.dktks.ba, E-mail: dktks.sekretar@gmail.com

Na osnovu člana 18. Zakona o udruženjima i fondacijama ("Službene novine Federacije BiH", broj 45/02), i člana 19. Statuta Udruženja "Društvo kemičara i tehnologa Kantona Sarajevo" Skupština Društva na sjednici održanoj dana 14.02.2015. godine, donijela je

ODLUKU O IMENOVANJU ČLANOVA

Nadzornog Odbora
Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

Član 1.

U Nadzorni odbor udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“ imenuju se :

1. Aida Šapčanin, predsjednik
2. Hajrudin Hajdar, član
3. Jozo Ćorić, član

Član 2.

Izabrani članovi Nadzornog odbora Udruženja su postali kandidati sa najvećim brojem glasova. Mandat članova Nadzornog odbora traje 2 godine.

Član 3.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-04/15

14. februar 2015.g

Sarajevo

Predsjednik Skupštine

Memić Mustafa

DKT KS
DRUŠTVO KEMIČARA I TEHNOLOGA
KANTONA SARAJEVO
BOSNA I HERCEGOVINA
Zmaja od Bosne 35, 71000 Sarajevo, Bosna i Hercegovina
Tel. +387 33 279 911
Web: www.dktks.ba, E-mail: dktks.sekretar@gmail.com

Na osnovu člana 18. Zakona o udruženjima i fondacijama ("Službene novine Federacije BiH", broj 45/02), i člana 19. Statuta Udruženja "Društvo kemičara i tehnologa Kantona Sarajevo" Skupština Društva na sjednici održanoj dana 14.02.2015. godine, donijela je

ODLUKU O IMENOVANJU ČLANOVA

Suda časti
Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

Član 1.

U Sud časti udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“ imenuju se:

1. Faiza Muštović-Biščević, član
2. Ismet Tahirović, član
3. Borivoj Galić, član
4. Nurudin Avdić, član
5. Mirza Nuhanović, član

Član 2.

Izabrani članovi Suda časti su postali kandidati sa najvećim brojem glasova. Mandat članova Suda časti traje 2 godine.

Član 3.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-05/15

14. februar 2015.g

Sarajevo

Predsjednik Skupštine

Memić Mustafa

DKT KS
DRUŠTVO KEMIČARA I TEHNOLOGA
KANTONA SARAJEVO
BOSNA I HERCEGOVINA
Zmaja od Bosne 35, 71000 Sarajevo, Bosna i Hercegovina
Tel. +387 33 279 911
Web: www.dktks.ba, E-mail: dktks.sekretar@gmail.com

Na osnovu člana 18. Zakona o udruženjima i fondacijama ("Službene novine Federacije BiH", broj 45/02), i člana 19. Statuta Udruženja "Društvo kemičara i tehnologa Kantona Sarajevo" Skupština Društva na sjednici održanoj dana 14.02.2015. godine, donijela je:

ODLUKU

o razrješenju i imenovanju lica za zastupanje i predstavljanje

Član 1.

Razrješavaju se dužnosti lica za zastupanje i predstavljanje udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“:

1. Harun Kurtagić, predsjednik Društva

2. Jelena Ostojić, član Upravnog odbora

3. Sabina Žero, član Upravnog odbora

Član 2.

Imenuju se lica za zastupanje i prestavljanje udruženja „Društvo kemičara i tehnologa Kantona Sarajevo:

1. Edhem Mulaosmanović, predsjednik Društva

2. Jelena Ostojić, član Upravnog odbora

3. Sabina Žero, član Upravnog odbora

Član 3.

Izabrana lica za zastupanje i predstavljanje su po članu 7. Statuta Udruženja DKTKS predsjednik Društva i dva odabrana člana Upravnog odbora. Mandat lica za zastupanje i predstavljanje traje 2 godine.

Član 4.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-06/15

14. februart 2015.g

Sarajevo

Predsjednik Skupštine

Memić Mustafa

DKT KS
DRUŠTVO KEMIČARA I TEHNOLOGA
KANTONA SARAJEVO
BOSNA I HERCEGOVINA
Zmaja od Bosne 35, 71000 Sarajevo, Bosna i Hercegovina
Tel. +387 33 279 911
Web: www.dktks.ba, E-mail: dktks.sekretar@gmail.com

Na osnovu člana 15. Zakona o udruženjima i fondacijama ("Službene novine Federacije BiH", broj 45/02), i članova 8.i 50. Statuta Udruženja "Društvo kemičara i tehnologa Kantona Sarajevo" Upravni odbor Društva na sjednici održanoj dana 07.05.2015. godine, donijela je

ODLUKU O FORMIRANJU SEKCIJA

Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

Član 1.

Upravni odbor je formirao sekcije slijedećih naziva:

1. Sekcija za organsku hemiju
2. Sekcija za analitičku hemiju
3. Sekcija za hemiju u industriji
4. Sekcija za nastavu
5. Sekcija za mlade
6. Sekcija za zaštitu životne sredine
7. Sekcija za fizikalnu hemiju i radiohemiju
8. Sekcija za hemiju u interdisciplinarnim naukama
9. Sekcija za nomenklaturu
10. Sekcija za elektrohemiju

Član 2.

Kao voditelji (predsjednici) navedenih sekcija, imenovane su sljedeće osobe koje su članovi Društva a bave se datom oblasti:

1. Lejla Klepo
2. Šaćira Mandal
3. Erdal Mršić
4. Ines Nuić
5. Zerina Bešić
6. Bojan Vujisić
7. Safija Herenda
8. Omer Mahmutović
9. Mirha Pazalja
10. Sanjin Gutić

DKT KS
DRUŠTVO KEMIČARA I TEHNOLOGA
KANTONA SARAJEVO
BOSNA I HERCEGOVINA
Zmaja od Bosne 35, 71000 Sarajevo, Bosna i Hercegovina
Tel. +387 33 279 911
Web: www.dktks.ba, E-mail: dktks.sekretar@gmail.com

Član 3.

Mandat voditelja sekcija traje 2 godine.

Član 4.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-07/15
07. maj 2015.g
Sarajevo

Predsjednik Upravnog odbora

Edhem Mulaosmanović

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).
6. *Extended Abstracts* – (about 2 typewritten pages) of Lectures given at international meetings.
7. *Technical Papers* – (about 10 typewritten pages) report on applications of an already described innovation. Typically, technical articles are not based on new experiments.

Reviewing the Manuscript

All contributors are evaluated according to the criteria of originality and quality of their scientific content, and only those deemed worthy will be accepted for publication. To facilitate the reviewing process, authors are encouraged to suggest three persons competent to review their manuscript. Such suggestions will be taken into consideration but not always accepted.

The Editor-In-Chief and Editors have the right to decline formal review of a manuscript when it is deemed that the manuscript is:

1. on a topic outside the scope of the Journal;
2. lacking technical merit;
3. of insufficient novelty for a wide international readership;
4. fragmentary and providing marginally incremental results; or
5. is poorly written.

Proofs

When a manuscript is ready for printing, the corresponding author will receive a PDF-formatted manuscript for proof reading, which should be returned to the journal within one week. Failure to do so will be taken as the authors are in agreement with any alteration which may have occurred during the preparation of the manuscript.

Copyright

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations.

Professional Ethics and Publication Policy

The journal expects the Editors, Referees and authors to adhere to the well-known standards of professional ethics. Authors are responsible for the factual accuracy of their contributions. Submission of the paper commits the author not to submit the same material elsewhere. Referees should act promptly. If certain circumstances preclude prompt attention to the manuscript at the time it is received, the non-received manuscript should be returned immediately to the Editor or the Referee should contact the Editor for possible delay of the report submission date. The Editor accepts full responsibility for his decisions on the manuscripts.

PREPARATION AND SUBMISSION OF MANUSCRIPT

Cover Letter

Manuscripts must be accompanied by a cover letter in which the type of the submitted manuscript. It should contain:

1. full name(s) of the author(s),
2. mailing address (address, phone and fax numbers, e-mail) of the author to whom correspondence should be addressed,
3. title of the paper (concise, without any abbreviations),
4. type of contribution,
5. a statement that the article is original and is currently not under consideration by any other journal or any other medium, including preprints, electronic journals and computer databases in the public domain, and
6. the names, full affiliation (department, institution, city and country), and
7. e-mail addresses of three potential Referees.

Contributors from Bosnia and Herzegovina should provide the name and full affiliation of at least one Referee from abroad.

Authors are fully encouraged to use ***Cover Letter Template*.**

Manuscript preparation

The submitted articles must be prepared with Word for Windows. Manuscripts should be typed in English (either standard British or American English, but consistent throughout) with 1.5 spacing (12 points Times New Roman; Greek letters in the character font Symbol) in A4 format leaving 2.5 cm for margins. Authors are fully encouraged to use ***Manuscript Template***.

All contributions should be written in a style that addresses a wider audience than papers in more specialized journals. Manuscripts with grammar or vocabulary deficiencies are disadvantaged during the scientific review process and, even if accepted, may be returned to the author to be rewritten in idiomatic English. The authors are requested to seek the assistance of competent English language expert, if necessary, to ensure their English is of a reasonable standard. The journal maintains its policy and takes the liberty of correcting the English of manuscripts scientifically accepted for publication.

Tables and figures and/or schemes should not be embedded in the manuscript but their position in the text indicated. In electronic version (Word.doc document) tables and figures and/or schemes should follow the text, each on a separate page. Please number all pages of the manuscript including separate lists of references, tables and figures with their captions.

IUPAC and International Union of Biochemistry and Molecular Biology recommendations for the naming of compounds should be followed.

SI units, or other permissible units, should be employed. The designation of physical quantities should be in Times New Roman font. In text, graphs, and tables, brackets should be used to separate the designation of a physical quantity from the unit. Please do not use the axes of graphs for additional explanations; these should be mentioned in the figure captions and/or the manuscript (example: "pressure at the inlet of the system, kPa" should be avoided).

Percents and *per mills*, although not being units in the same sense as the units of dimensioned quantities, can be treated as such. Unit symbols should never be modified (for instance: w/w %, vol.%, mol.%) but the quantity measured has to be named, *e.g.* mass fraction, w=95 %; amount (mole) fraction, x=20 %.

Latin words, as well as the names of species, should be in *italic*, as for example: *i.e., e.g., in vivo, ibid, Artemisia annua* L., *etc*. The branching of organic compound should also be indicated in *italic*, for example, *n*-butanol, *tert*-butanol, *etc*.

Decimal numbers must have decimal points and not commas in the text (except in the Bosnian/Croatian/Serbian abstract), tables and axis labels in graphical presentations of results. Thousands are separated, if at all, by a comma and not a point.

Structure of the Manuscript

The manuscript must contain, each on a separate page, the title page, abstract in English, (abstract in Bosnian/Croatian/Serbian), graphical abstract (optional), main text,

list of references, tables (each table separately), illustrations (each separately), and legends to illustrations (all on the same page).

1. ***Title page*** must contain: the title of the paper (bold letters), full name(s) of the author(s), full mailing addresses of all authors (italic), keywords (up to 6), the phone and fax numbers and the e-mail address of the corresponding author.
2. A one-paragraph ***abstract*** written of 150–200 words in an impersonal form indicating the aims of the work, the main results and conclusions should be given and clearly set off from the text. Domestic authors should also submit, on a separate page, a Summary/Sažetak. For authors outside Bosnia and Herzegovina, the Editorial Board will provide a Bosnian/Croatian/Serbian translation of their English abstract.
3. Authors are encouraged to submit a ***graphical abstract*** that describes the subject matter of the paper. It should contain the title of the paper, full name(s) of the author(s), and graphic that should be no larger than 11 cm wide by 5 cm tall. Authors are fully encouraged to use ***Graphical Abstract Template.***
4. ***Main text*** should have the following form:
   - ***Introduction*** should include the aim of the research and a concise description of background information and related studies directly connected to the paper.
   - ***Experimental*** section should give the purity and source of all employed materials, as well as details of the instruments used. The employed methods should be described in sufficient detail to enable experienced persons to repeat them. Standard procedures should be referenced and only modifications described in detail.
   - ***Results and Discussion*** should include concisely presented results and their significance discussed and compared to relevant literature data. The results and discussion may be combined or kept separate.
   - The inclusion of a ***Conclusion*** section, which briefly summarizes the principal conclusions, is highly recommended.
   - ***Acknowledgement*** (optional).
   - Please ensure that every ***reference*** cited in the text is also present in the reference list (and *vice versa*). Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either "Unpublished results" or "Personal communication" Citation of a reference as "in press" implies that the item has been accepted for publication. As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. No more than 30 references should be cited in your manuscript.

     In the text refer to the author's name (without initials) and year of publication (e.g. "Steventon, Donald and Gladden (1994) studied the effects..." or "...similar to values reported by others (Anderson, Douglas, Morrison, *et al.*, 1990)..."). Type the names of the first three authors at first citation. At subsequent citations use

first author *et al.* The list of references should be arranged alphabetically by authors' names and should be as full as possible, listing all authors, the full title of articles and journals, publisher and year.

Examples of ***reference style***:

a) Reference to a journal publication:
Warren, J. J., Tronic, T. A., Mayer, J. M. (2010). Termochemistry of proton-coupled electron transfer reagents and its implications. *Chemical Reviews*, *110 (12)*, 6961-7001.

b) Reference to a book:
Corey, E. J., Kurti, L. (2010). *Enantioselective chemical synthesis*. (1st Ed.) Direct Book Publishing, LLC.

c) Reference to a chapter in an edited book:
Moody, J. R., Beck II, C. M. (1997). Sample preparation in analytical chemistry. In Setlle, F. A. (Ed.), *Handbook of instrumental techniques for analytical chemistry*. (p.p. 55-72). Prentice Hall.

d) Reference to a proceeding:
Seliskar, C. J., Heineman, W.R., Shi, Y., Slaterbeck, A.F., Aryal, S., Ridgway, T.H., Nevin, J.H. (1997). *New spectroelectrochemical sensor*, in Proceedings of 37th Conference of Analytical Chemistry in Energy and Technology, Gatlinburg, Tenesee, USA, p.p. 8-11.

e) Patents:
Healey, P.J., Wright, S.M., Viltro, L.J., (2004). *Method and apparatus for the selection of oral care chemistry*, The Procter & Gamble Company Intellectual Property Division, (No.US 2004/0018475 A1).

f) Chemical Abstracts:
Habeger, C. F., Linhart, R. V., Adair, J. H. (1995). Adhesion to model surfaces in a flow through system. *Chemical Abstracts,* CA 124:25135.

g) Standards:
ISO 4790:1992. (2008). *Glass-to-glass sealings - Determination of stresses*.

h) Websites:
Chemical Abstract Service, www.cas.org, (18/12/2010).

- ***Tables*** are part of the text but must be given on separate pages, together with their captions. The tables should be numbered consequently in Latin numbers. Quantities should be separated from units by brackets. Footnotes to tables, in size 10 font, are to be indicated consequently (line-by-line) in superscript letters. Tables should be prepared with the aid of the Word table function, without vertical lines. Table columns must not be formatted using multiple spaces. Table rows must not be formatted using Carriage returns (enter key; ↵ key). Tables should not be incorporated as graphical objects.
- ***Figures and/or Schemes*** (in high resolution) should follow the captions, each on a separate page of the manuscript. High resolution illustrations in TIF or EPS format (JPG format is acceptable for colour and greyscale photos, only) must be uploaded as a separate archived (.zip or .rar) file.

Figures and/or Schemes should be prepared according to the artwork instructions.

- ***Mathematical and chemical equations*** must be numbered, Arabic numbers, consecutively in parenthesis at the end of the line. All equations should be embedded in the text except when they contain graphical elements (tables, figures, schemes and formulae). Complex equations (fractions, inegrals, matrix…) should be prepared with the aid of the Word Equation editor.

Artwork Instructions

Journal accepts only TIF or EPS formats, as well as JPEG format (only for colour and greyscale photographs) for electronic artwork and graphic files. MS files (Word, PowerPoint, Excel, Visio) are NOT acceptable. Generally, scanned instrument data sheets should be avoided. Authors are responsible for the quality of their submitted artwork.

Image quality: keep figures as simple as possible for clarity - avoid unnecessary complexity, colouring and excessive detail. Images should be of sufficient quality for the printed version, i.e. 300 dpi minimum.

Image size: illustrations should be submitted at its *final size* (8 cm for single column width or 17 cm for double column width) so that neither reduction nor enlargement is required.

Photographs: please provide either high quality digital images (250 dpi resolution) or original prints. Computer print-outs or photocopies will not reproduce well enough for publication. Colour photographs rarely reproduce satisfactorily in black and white.

The facility exist for color reproduction, however the inclusion of color photographs in a paper must be agreed with Editor in advance.

Reporting analytical and spectral data

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).

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

Submission Checklist

The following list will be useful during the final checking of an article prior to sending it to the journal
for review:

- E-mail address for corresponding author,
- Full postal address,
- Telephone and fax numbers,
- All figure captions,
- All tables (including title, description, footnotes),
- Manuscript has been "spellchecked" and "grammar-checked",
- References are in the correct format for the journal,
- All references mentioned in the Reference list are cited in the text, and *vice versa*.

Submissions

Submissions should be directed to the Editor by e-mail: ***glasnik@pmf.unsa.ba***, or ***glasnikhtbh@gmail.com***. All manuscripts will be acknowledged on receipt (by e-mail) and given a reference number, which should be quoted in all subsequent correspondence.

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

Print ISSN: 0367-4444
Online ISSN: 2232-7266

Zmaja od Bosne 33-35, BA-Sarajevo
Bosnia and Herzegovina
Phone: +387-33-279-918
Fax: +387-33-649-359
E-mail: glasnik@pmf.unsa.ba
glasnikhtbh@gmail.com

Sponsors

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

Print ISSN: 0367-4444
Online ISSN: 2232-7266

Zmaja od Bosne 33-35, BA-Sarajevo
Bosnia and Herzegovina
Phone: +387-33-279-918
Fax: +387-33-649-359
E-mail: glasnik@pmf.unsa.ba
glasnikhtbh@gmail.com

www.elektroprivreda.ba/stranica/te-kakanj

Zahvaljujemo se **Federalnom ministarstvu obrazovanja i nauke** na finansijskoj pomoći za izdavanje ovog broja ***Glasnika hemičara i tehnologa Bosne i Hercegovine***.

Redakcija GLASNIKA-a