Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2017

49

15-18

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

UDC: _____

Original Scientific Paper

Antioxidant activity of fruits and vegetables commonly used in everyday diet in Bosnia and Herzegovina

Šapčanin, A.[a*], Salihović, M.[a], Uzunović, A.[b], Osmanović, A.[a], Špirtović-Halilović, S.[a], Pehlić, E.[c], Jančan, G.[d]

[a] *Faculty of Pharmacy, University of Sarajevo, Zmaja od Bosne 8, 71 000 Sarajevo, BiH*
[b] *Agency for medicinal products and medical devices of Bosnia and Herzegovina, Marsala Tita 9, 71000 Sarajevo, BiH*
[c] *Faculty of Biotechnical Sciences, University of Bihac, Luke Marjanovića bb, 77 000 Bihac, BiH*
[d] *Chemilab d.o.o., Dunajska cesta 238E, 1000 Ljubljana, Slovenia*

Article info
Received: 21/09/2017
Accepted: 10/11/2017

Keywords:
Fruits
Vegetables
Total Phenols
Total Flavonoids
Antioxidant Activity

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

Abstract: Total phenols, flavonoids and their ratios, as well as antioxidant activity of selected fruits (banana, apple, plum, raspberry, strawberry, orange and peach) and vegetables (broccoli, onion, spinach, red cabbage, tomato, lettuce, leek and cauliflower) were determined by spectrophotometric methods. The antioxidant activity of the selected fruits and vegetables was evaluated by TEAC and FRAP methods. Results showed that total phenol content (mg GAE/g) in fruits was in the range from 0.38 to 3.30, while in vegetables it was in the range from 0.22 to 1.58. Total flavonoid content (mg GAE/g) in fruits was in the range from 0.21 to 2.20, while in vegetables it was in the range from 0.06 to 0.98. The highest ratio of total phenolic and flavonoid compounds was observed for strawberry and tomato, while the lowest one was for lettuce. Antioxidant activity by TEAC and FRAP methods showed the highest results for strawberries and red cabbage. Investigated fruits and vegetables consumed on a daily basis in the households in Bosnia and Herzegovina, can be considered as an exceptionally good source of natural phenols and flavonoids. Investigated fruits and vegetables are often used fresh in homemade dishes so that phenols and flavonoids are not destroyed by thermal processing and are therefore more available and more potent as antioxidants in vivo.

INTRODUCTION

Consumption of fruits and vegetables in large quantities has been considered crucial in human health prevention against numerous diseases - cancer, diabetes, neurodegenerative and cardiovascular diseases. Currently, the opinion is that protective properties of many foods come from low molecular antioxidants present in such foods which protect their structures from oxidative damage (Wang and Lin, 2000). Aforementioned risk-reducing effect for many diseases is not a result of the activity of an individual antioxidant like α-tocopherol, ascorbic acid or β-carotene, but could also be a result of antioxidant compounds not yet found, or of a synergy of several different antioxidants present in fruits and vegetables (Teow, Truong, McFeeters, et al., 2007). Research on macromolecules (DNA, nucleotides, proteins) being damaged by free radicals showed that diets enriched in additional amounts of fruits rich with β-carotene, tocopherols and ascorbic acid, had limited influence on inhibiting oxidation processes (Day, Seymour, Pitts, et al., 2009). However, research showed that consuming different sorts of fruits and vegetables contributes to lowering free radical processes, probably because they

contain great amounts of non-vitamin antioxidants, such as polyphenols and anthocyanins (Zhishen, Mengcheng, Jianming, 1999; Lichtenthäler, Rodrigues, Maia, Papagiannopoulos, et al., 2005). Presence of phenolic compounds in food is of great importance because of the demonstrated antioxidant activity, prevention of cancerogenesis and mutagenesis (Re, Pellegrini, Proteggente, et al., 1999). Polyphenols have an important role in maintaining sensory properties of food during thermal processing. Knowledge of polyphenolic composition of the food and its change during storage and processing is essential in order to ensure a good food quality. Anthocyanins are responsible for red, blue and purple colors of many sorts of food, including fruits and vegetables, while flavonols and tannins are involved in the taste formation (astringent) and bitterness (Hangun-Balkir, McKenney, 2012). Everything aforementioned makes antioxidants an increasingly interesting research topic.

MATERIALS AND METHODS

Total phenols, flavonoids and their ratio, as well as the antioxidant activity of samples of the selected fruits (banana, apple, plum, raspberry, strawberry, orange and peach) and vegetables (broccoli, onion, spinach, red cabbage, tomato, lettuce, leek and cauliflower) were determined by spectrophotometric methods. The antioxidant activity of the selected fruits and vegetables was evaluated by TEAC and FRAP methods.

Sample preparation

Samples of the tested fruits and vegetables (500 mg each) were powdered in a mortar, homogenized by addition of ethanol (80% v/v), and then centrifuged (15000 rpm, 20 minutes) at temperature of 4 °C. Supernatants obtained by such processing were used for determination of phenols and flavonoids as well as of the antioxidant activity of the extracts.

Total phenolic content determination

Total phenols (TP) were determined spectrophotometrically using *Folin-Ciocalteu* reagent (Waterhouse, 2002). Total phenolic content in individual samples was determined by extrapolation from calibration curve obtained by measuring apsorption of gallic acid solutions of known concentrations (0 – 2.0 mg/mL) and was expressed in milligrams of gallic acid equivalent by gram of fresh weight of the sample (mg GAE/g).

Total flavonoid content determination

Total flavonoid content was determined by spectrophotometric method based on forming yellow flavonoid complex with aluminum (Willet, 2002). Absorbtion was measured at 420 nm. Quercetin was used as the standard. Total flavonoid content in the individual samples was determined by extrapolation from calibration curve obtained by measuring absorption of quercetin ethanolic solutions of known concentrations (0 – 30 mg/mL) and was expressed in milligrams of quercetin equivalent by gram of fresh weight of the sample (mg QuE/g).

Oxidation potential determination by TEAC method

TEAC test (Trolox Equivalent Antioxidant Capacity) is a method of the antioxidant activity determination which measures the ability of the sample to catch/neutralize $ABTS^{\cdot+}$ (2,2'-azino-bis(3-ethylbenzothiazolin-6-sulfonic acid) radical action. $ABTS^{\cdot+}$ radical is generated directly into a stable form using potassium persulfate as an oxidation agent. Such formed radical absorbs at 732 nm, and after mixing with an antioxidant in the reactive medium, the absorbtion drop appears at that wavelength as a result of a reaction between the radical and the antioxidant. Trolox is usually used as the standard (watersoluble form of vitamin E) therefore the name of the method is TEAC (Trolox Equivalent Antioxidant Capacity), and the results are expressed as Trolox equivalents, or a Trolox solution concentration (mmol/mL) with the equivalent antioxidant activity as 1.0 mmol/mL solution of the tested compound (Prior and Schaich, 2005). The results of the samples testing are expressed by equation of calibration diagram as micromols of Trolox equivalent by gram of fresh weight of the sample (μmol Trolox/g).

Reduction potential determination by FRAP method

This method (Benzie and Strain, 1996) is based on reduction of colorless iron(III)-tripyridyltriazine (Fe^{3+} - TPTZ) complex into an intensively blue-colored fero form (Fe^{2+}). The antioxidant activity of the tested samples is determined by spectrophotometric measurement of absorption at 593 nm. The calibration curve for FRAP reagent is prepared by mixing acetate buffer (300 mmol/L, pH=3.6), TPTZ reagent (10 mmol/L in 40 mmol/L HCl) and $FeCl_3x6H_2O$ (20 mmol) in ratio 10:1:1. In each of the test tubes, 3 mL of FRAP reagent was measured and 0.1 mL of FeSO4x7H2O standard solution of 0.2-1 mmol/mL was added. Absorption was monitored at 593 nm compared to a blind test (3 mL of FRAP reagent and 0.1 mL of water). The concentration (mmol/mL) of Fe^{2+} in the sample was determined from the calibration curve equation and was converted to micromols of the exctract (μmol Fe^{2+}/g), representing the FRAP value.

RESULTS AND DISCUSSION

The total phenolic (TP) and total flavonoid (TF) contents, as well as their ratios in ethanolic extracts of the tested fruits and vegetables, were determined by spectrophotometry and the results are shown in Tables 1 and 2.

Table 1. The content of total phenols (TP) and flavonoids (TF) and their ratio (TP/TF) in fruit extracts

Fruit	TP (mg GAE/g)	TF (mg QuE/g)	TP/TF
Banana	0.38	0.21	1.727
Apple	0.48	0.39	1.237
Plum	3.20	**2.20**	1.457
Raspberry	2.28	1.02	2.236
Strawberry	**3.30**	0.97	**3.409**
Orange	1.26	0.76	1.658
Peach	0.38	0.21	1.818

Table 2. The content of total phenols (TP) and flavonoids (TF) and their ratio (TP/TF) in vegetable extracts

Vegetables	TP (mg GAE/g)	TF (mg QuE/g)	TP/TF
Broccoli	1.28	**0.98**	1.306
Onion	0.88	0.60	1.467
Spinach	0.72	0.32	2.250
Red cabbage	**1.58**	0.45	3.511
Tomato	0.30	0.06	**5.000**
Lettuce	0.14	0.36	0.389
Leek	0.22	0.75	0.293
Cauliflower	0.33	0.42	0.786

From the results shown in Tables 1 and 2, among the tested samples, strawberries and red cabbage are the richest source of total phenols, while plum and leek are the richest source of total flavonoids. Total phenolic content (mg GAE/g) in the fruits ranged from 0.38 to 3.30, while in the vegetables it ranged from 0.220 to 1.58 . Total flavonoid content (mg GAE/g) in the fruits ranged from 0.21 to 2.20, while in the vegetables it ranged from 0.06 to 0.98. The highest ratio of the total phenolic and flavonoid compounds was observed for strawberry and tomato, while the lowest was for lettuce.

TEAC and FRAP values of ethanolic extracts for the tested fruits and vegetables are given in Tables 3 and 4.

Table 3. TEAC and FRAP values of fruit extracts

Fruit	TEAC (μmol Trolox/g)	FRAP (μmol Fe^{2+}/g)
Banana	1.81	1.64
Apple	3.43	3.94
Plum	18.25	20.57
Raspberry	18.46	23.25
Strawberry	**25.90**	**33.50**
Orange	8.49	11.81
Peach	1.95	2.02

Table 4. TEAC and FRAP values of vegetable extracts.

Vegetables	TEAC (μmol Trolox/g)	FRAP (μmol Fe^{2+}/g)
Broccoli	6.48	8.33
Onion	5.32	3.69
Spinach	7.57	10.09
Red cabbage	**13.77**	**18.70**
Tomato	2.55	3.44
Lettuce	1.71	1.24
Leek	2.40	1.60
Cauliflower	2.95	2.59

According to the results presented in Table 3 (TEAC and FRAP for the ethanolic fruit extracts) the highest radical scavenging capacity by TEAC method was determined in the strawberry extract, while the lowest was in the banana extract. Also, Table 3 shows that the highest FRAP value, meaning also the best reduction ability, was observed in the strawberry extract, while the lowest was in the banana extract. Table 4 shows that the highest FRAP and TEAC values was observed in the red cabbage extract, while the lowest was in the lettuce extract.

The research has shown that many sorts of fruits contain higher amounts of phenolic compounds. The research on red fruits showed that an average phenolic content was in the range between 1.9 and 7.2 mg per 1 g of fresh fruit (Jakobek, Šeruga, Novak, et al., 2007). However, our research has shown that strawberry, plum and raspberry contain higher amounts of phenols and flavonoids than citrus fruits. Also, some research showed that different sorts of vegetables contain lower amounts of total phenols and flavonoids than citruses (Katalinic, Miloš, Kulisic et al., 2006). Our results support these findings.

The research revealed that TEAC and FRAP tests show differences in the antiradical effect which depends on the phenol content in fruits and vegetables; consequently, the higher total phenolic content, the stronger the antiradical effect it is (Katalinic, Miloš, Kulisic et al., 2006). Our results are in good accordance with other studies.

Different investigations have shown a relationship between the total phenolic contents and the antioxidative activity of the fruits, plants and vegetables (Deighton, Brennan, Finn, et al., 2000; Abdille, Singh, Jayaprakasha, et al., 2005; Vinson, Hao, Su, et al., 1998). The chemical composition and chemical components in plant extracts are important factors governing the efficacy of natural antioxidants. The antioxidant activity of an extract could not be explained on the basis of their phenolic content, which also requires their chemical characterization (Halliwell, Gutteridge, 2015).

CONCLUSIONS

For the purpose of the bioactive content determination in the tested fruits and vegetables, the total phenolic and flavonoid contents were determined using Folin-Ciocalteau reagent, and the antioxidant capacity was determined by FRAP and TEAC methods. Based on the obtained results, the conclusions can be drawn as follows.

Investigated fruits and vegetables, commonly used in everyday consumption in Bosnia and Herzegovina, can be considered as an exceptionally good source of natural phenols and flavonoids.

Investigated fruits and vegetables are often used fresh in homemade dishes; that is why phenols and flavonoids are not destroyed by thermal processing and are therefore more available and more potent as antioxidants *in vivo*.

Therefore, in order to ensure optimal nutrition, health, and well-being, consumers should obtain their nutrients, antioxidants, bioactive compounds, or phytochemicals from their balanced diet based on a wide variety of fruits, vegetables, and other plant foods.

Further research on the health benefits of the investigated plant material is necessary.

Acknowledgement

This work was supported by the Federal Ministry of Education and Science in Bosnia and Herzegovina and was carried out within the project „Investigation of the antioxidative status of different plants used in everyday nutrition and obtained from Bosnian markets“ (Grant no. 0101-7552-17/15, dated 14.12.2015).

REFERENCE

Abdille, M. H., Singh, R. P., Jayaprakasha, G. K., Jena, B. S. (2005). Antioxidant activity of the extracts from Dillenia indica fruits. *Food chemistry*, *90*(4), 891-896.

Benzie, I. F., Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem*. 239, 70-76.

Day, L., Seymour, R. B., Pitts, K. F., Konczak, I., Lundin, L. (2009). Incorporation of functional ingredients into foods. *Trends in Food Science & Technology*, *20*(9), 388-39.

Deighton, N., Brennan, R., Finn, C., Davies, H. V. (2000). Antioxidant properties of domesticated and wild Rubus species. *Journal of the Science of Food and Agriculture*, *80*(9), 1307-1313.

Halliwell, B., Gutteridge, J. M. (2015). *Free radicals in biology and medicine*. Oxford University Press, USA.

Hangun-Balkir, Y., McKenney, M. L. (2012). Determination of antioxidant activities of berries and resveratrol. *Green Chemistry Letters and Reviews*, *5*(2), 147-153

Jakobek, L., Šeruga, M., Novak, I., Medvidović-Kosanović, M. (2007). Flavonols, phenolic acids and antioxidant activity of some red fruits. *Deutsche Lebensmittel-Rundschau*, *103*(8), 369-378.

Katalinic, V., Milos, M., Kulisic, T., Jukic, M. (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food chemistry*, *94*(4), 550-557.

Lichtenthäler, R., Rodrigues, R. B., Maia, J. G. S., Papagiannopoulos, M., Fabricius, H., & Marx, F. (2005). Total oxidant scavenging capacities of Euterpe oleracea Mart.(Acai) fruits. *International Journal of Food Sciences and Nutrition*, *56*(1), 53-64.

Prior, R. L., Wu, X., Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of agricultural and food chemistry*, *53*(10), 4290-4302.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay.*Free radical biology and medicine*,*26*(9), 1231-1237.

Teow, C., Truong, V. D., McFeeters, R., Thompson, R., Pecota, K., Yencho, G. (2007). Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colours. *Food Chem. 103*, 829-838.

Vinson, J. A., Hao, Y., Su, X., Zubik, L. (1998). Phenol antioxidant quantity and quality in foods: vegetables. *Journal of Agricultural and Food Chemistry*, *46*(9), 3630-3634..

Wang, S. Y., Lin, H. S. (2000). Antioxidant activity in Fruits and Leaves of Blackberry, Raspberry and Strawberry Varies with Cultivar and Developmental Stage. *J. Agric. Food Chem. 48*, 140-146.

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

Willett, W. C. (2002). Balancing life-style and genomics research for disease prevention. *Science*, *296*(5568), 695-698.

Zhishen, J., Mengcheng, T., Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food chemistry*, *64*(4), 555-559.

Summary/Sažetak

Ukupni fenoli, flavonoidi i njihov odnos, te antioksidativna aktivnost uzoraka odabranog voća (banana, jabuka, šljiva, malina, jagoda, naranča i breskva) i povrća (brokula, crveni luk, špinat, crveni kupus, paradajz, salata, prasa i karfiol) su određivani spektrofotometrijskim metodama. Antioksidativne aktivnosti odabranog voća i povrća određene su TEAC i FRAP metodama. Rezultati su pokazali da se sadržaj ukupnih fenola (mg GAE/g) u voću kreće u rasponu od 0.38 – 3.30, a u povrću u rasponu od: 0.22 – 1.58. Sadržaj ukupnih flavonoida (mg GAE/g) u voću kreće se u rasponu od: 0.21 – 2.20, a u povrću u rasponu od: 0.06 – 0.98. Najveći odnos ukupnih fenola i ukupnih flavonoida bio je za jagodu i paradajz, a najmanji za salatu. Za dio ispitivanja koji se odnosio na antioksidativnu aktivnost TEAC i FRAP metodom je utvrđeno da su najveću antioksidativnu aktivnost pokazale jagode i crveni kupus. Može se konstatovati da bi se ispitivane vrste voća i povrća, koje se najčešće konzumiraju u bosanskohercegovačkim domaćinstvima, mogu smatrati izuzetno dobrim izvorom prirodnih fenola i flavonoida.Ispitivano voće i povrće se često upotrebljava svježe u domaćim receptima i radi toga se fenoli i flavonoidi ne uništavaju termičkom obradom i stoga su dostupniji i potentniji kao antioksidanti in vivo.