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The Influence of Total Phenols Content on Antioxidant Capacity in the Whole Grain Extracts

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Abstract

Positive health effects of integral cereals consuming is attributed to bioactive substances. This research is conducted to test antioxidant water extract capacity of different kinds of cereals, compared to the values of total phenols. The total antioxidant capacity was estimated by FRAP assay. The total phenolic content was measured by Folin-Ciocalteau assay. Antioxidant capacity amounted 50,04–385,71 μmol Fe^{II}/L of extract, and values of total phenols 2,95–20,35 mg GA equiv/L of extract on 20°C. The increase of temperature reaction (40°C) resulted in increased content of total phenols in extract and higher value of antioxidant capacity in all extracts. There was significant linear corelation between total phenolic content and FRAP.

Keywords: Whole grain, water extract, antioxidant capacity, FRAP assay, total phenols

1. Introduction

Research in the area of preventive medecine shows that functional nutrition plays the key role in reducing the risk factor of certain chronic diseases. Consumation of integral cereals is connected to: risk reduction of cardiovascular system diseases and disorders (Liu et al, 1999; Jacobs et al, 1998), prevention of some types of cancer (Adlercreutz, 1990) and diabetes (Salmeron et al, 1997) also hypertension (Ascherio et al, 1992). Whole grains contain complex carbohydrates (galacto and fructooligosaccharides), resistant starch, dietary fibres (β-glucan and arboxilan), vitamines, minerals, phytoestrogenes, microelements and polyphenols (Charalampopulos et al, 2002; Anderson, 1995). Generaly, integral cereals are rich source of antioxidant compounds which inhibit oxidational stress (Slavin, 1994). Oxidational stress can be the result of normal metabolic activity or enviromental factors. The balance antioxidants-prooxidants is dinamic, and in human organism it is moved towards oxidation which is essential for energy creation (Rumenjak, 2005). Antioxidants through their activity contribute to the protection of organism against oxidational stress. Insufficient entry of antioxidants in human organism can lead to the biological structure damages: DNA, lipids and proteins (Gutteridge and Halliwell, 1994; Papas, 1999).

Phenolic compounds are secondary metabolites which synthesize in plants. They posses biological properities such as: antioxidant, antiapoptosis, anti-aging, anticarcinogen, anti-inflammation, anti-artherosclerosis, cardiovascular protection, improvement of the endothelialfunction, as well as inhibition of angiogenesis and cell proliferation activity. Most of these biological actions have been attributed to their intrinisc reducing capabilities (Han, 2007).

Plant foods have phenolic compounds, which affect their: apperance, taste, odor and oxidative stability. In cereal grains, these compounds are located mainly in the pericarp (Naczk et al, 2004). The major phenolic acids in cereals are ferulic and p-coumaric acids (Hahn et al, 1983; Holtekjolen et al, 2006; Mattila et al, 2005; Zhou et al, 2004). Anthocyanins are water-soluble pigments mostly studied in cereals (Yao et al, 2004).

The aim of the present study was to compare total phenolic and antioxidant activity levels in the whole grain water extracts, that comes from the area of Bosnia and Herzegovina (except rice-Italy). Total antioxidant capacity has been determined using ferric-reducing ability of plasma assay (FRAP) of Benzie and Strain (Benzie and Strain, 1996). The efficiency of extracted phenolics was evaluated using the phenol antioxidant coeficient (PAC) (Katalinic et al, 2006).

2. Experimental

2.1. Chemicals, Reagents and Glassware

All chemicals and reagents were of analytical grade and were purchased from: (Fluka-Switzerland: 2,4,6-tri[2- pyridyl]-s-triazine), (Merck-Germany: galic acid), (Kemika-Croatia: Folin-Ciocalteu reagent) and (Semikem-Sarajevo: chloric acid 37% p.a.; ferrous sulphate heptahydrate; ferric chloride hexahydrate; sodium acetate trihydrate; acetic acid (conc.); sodium carbonate).

Cereal samples are gathered in the area of Bosnia and Herzegovina (except rice-Italy). All whole grain samples were milled in a coffee grinder to a fine powder. Solutions, 5% (w/v), milled integral cereal samples are subjected to half an hour extraction on 20°C and 40°C. The extracts were filtered and the liquid portions and analyzed for the total phenolic content and antioxidant capacity. All glassware were rinsed successively with detergent and distilled water three times prior to use.

2.2. Spectrophotometric Measurements

Spectrophotometric measurements were performed by Cecil CE 2021 UV-VIS spectrophotometer.

2.2.1. Determination of Antioxidant Capacity

FRAP assay measures the change in absorbance at 593 nm owing to the formation of blue colored Fe^{II}-tripyridyltriazine compound from colorless oxidzed Fe^{III} form by the action of electron donating antioxidants. Standard curve was prepared using different concentrations (100-1000 µmol/L) $FeSO_4 \times 7H_2O$. All solutions were used on the day of preparation. In the FRAP assay the antioxidant efficiency of the antioxidant under the test was calculated with reference to the reaction signal given by an Fe^{II}-solution of known concentration, this representing a one electron exchange reaction. The results were expressed in µmol Fe^{II}/L of extract. Data presented are average of three replications.

2.2.2. Determination of Total Phenolic Content

The total phenolic content of extracts was determined using to the Folin-Ciocalteu method (Singelton et al. 1999). The extracts were oxidized with Folin-Ciocalteu reagent, and the reaction was neutralized with sodium carbonate. The absorbance of the resulting blue color was measured at 760 nm after 60 min. Using galic acid as standard total phenolic content (standard curve was prepared using concentratons 2,5-50 mg/L) was expresed as mg GA equivalent/L of extract. Data reported of three replications.

2.3. Statistical Analysis

The direction and magnitude of corelation between variables was done using analysis of variance (ARCUS QUICKSTADT BIOMEDICAL, ADDISON WESLEY) and quantified by the correlation factor „r“. The P-values less than 0,001 were considered statistically significant.

3. Results and Discussion

3.1. Total Content of Phenols in the Whole Grain Extracts

In the last decade a number of publications have been published in which antioxidant capacity of plant material, so as antioxidant characteristics of phenol compounds are tested, through different methods (Velioglu et al, 1998; Miller et al, 2000; Halvorsen et al, 2002; Javanmardi et al, 2003). Because of this it is difficult to compare final results, even though there are the same plant species.

Conducted research shows that values of total phenolic compounds in extracts vary from 2,95-20,35 mg GA/L of extracts on 20ºC. Higher temperature (40ºC) affects the better extraction of total phenols (Table 2), and given values are in average 4,29-30,65 mg GA/L. The higest concentration is measured in buckwheat extract, followed by rye, oats, barley, corn, wheat, and rice, respectively. The research of total phenols in earlier published papers differ in preparation method of samples (solvent selection, extraction time and temperature). The final, values vary.

However, if we compare values of total phenolic compounds in different types of cereals, it can be concluded that content of total phenols in rice is the smallest. Brown rice is richer in phenols than white rice. It has been determined that buckwheat has very good antioxidant characteristics (Zielinski and Kozlowska, 2000; Adom and Liu, 2002). Some types of cereals are sources of large number of different phenolic compounds. Number of hydroxylic groups in phenolic compounds, so as their spatial orientation are proportional to molar response of this method (Frankel et al, 1995). This can be the reason for differences in values of total phenols in particular types of cereals.

3.2. Total antioxidant Capacity in the Whole Grain Extracts

FRAP-method was initialy developed to assay plasma antioxidant capacity, but can be used to measure the antioxidant capacity from a wide range of biological samples and pure compounds (Ghiselli et al, 1998; Ou et al, 2002). In this study, we used FRAP assay because it is quick and simple to perform, and reaction is reproducible and linearly related to the molar concentration of the antioxidants.

As shown in Table 1, there diferences in total antioxidant capacity average 50,5 - 385,71 µmol Fe^{II}/L of extract on 20°C. Values of antioxidant capacity in extracts prepared on higher temperature (40°C) are increased and amount to 64,28 - 453,8 µmol Fe^{II}/L of extract (Table 2). The highest antioxidant capacity is measured in buckwheat extract, then follow: rye > oats > barley > corn > wheat > rice. The significant linear correlation (correlation coefficient „r"= 0,9956; 95% confidence interval: 0,9875 - 0,9984; coefficient of determination „r^2" = 0,9912; p-value < 0,0001) was confirmed between total phenolics and related FRAP of grain extracts on 20° C.

Linear correlation between the amount of total phenols and antioxidant capacity (FRAP) is found in extracts prepared on temperature of 40° C. Correlation coefficient „r"= 0,9871; 95% confidence interval: 0,9638 - 0,9954. Coefficient of determination „r^2" = 0,9745; p-value < 0,0001 is considered extremely significant. Earlier researches confirm the correlation of tested parameters (Dykes and Rooney, 2007; . Emmons et al, 1999; Guajardo-Flores et al, 2006)

Table 1: The total phenolic content and related antioxidant capacity in the whole grain extract on 20°C

Type of cereals and origin	Total phenolics (mg GA/L)	FRAP (µmolFe^{II}/L)	PAC[a]
Buckwheat (Mrkonjic Grad,B&H)	20,35	385,71	3,23
Rye (Velika Kladusa, B&H)	18	338,12	3,19
Oats (Gnojnica-Lukavac, B&H)	17,1	328,59	3,26
Oats (Mrazovac-Buzim,B&H)	16,25	321,92	3,36
Oats (Velika Kladusa, B&H)	13,85	282,35	3,46
Barley (Velika Kladusa,B&H)	13,56	279,4	3,5
Barley (Mrazovac-Buzim, B&H)	13,3	274,93	3,51
Barley (Gnojnica-Lukavac, B&H)	11,69	238,09	3,46
Corn (Toplice, B&H)	11,29	233,35	3,51
Corn (Si Selo-Tuzla, B&H)	10,17	210,02	3,5
Corn (Srebrenik, B&H)	9,82	202,12	3,49
Wheat (Ljusine-Bos.Otoka,B&H)	9,26	190,26	3,49
Wheat (Velika Kladusa, B&H)	8,46	173,28	3,47
Wheat (Bos.Krupa, B&H)	6,48	116,45	3,05
Brown rice (Italy)	7,48	144,78	3,29
White rice (Italy)	2,95	50,05	2,88

[a]PAC- Phenol antioxidant coefficient, calculated as ratio FRAP (µmol/L) / total phenolic (µmol GA/L)

Figure 1: Linear correlation between the amount of total phenols and antioxidant capacity (FRAP) in the extracts on 20°C

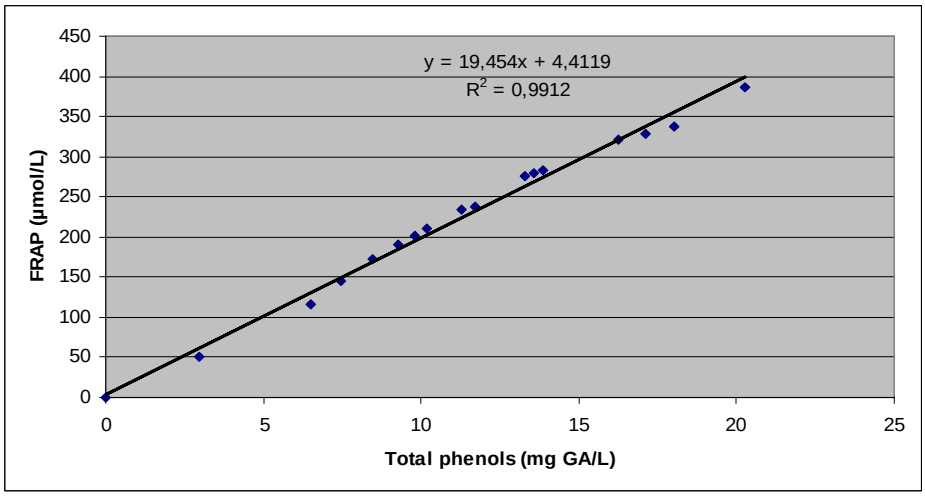
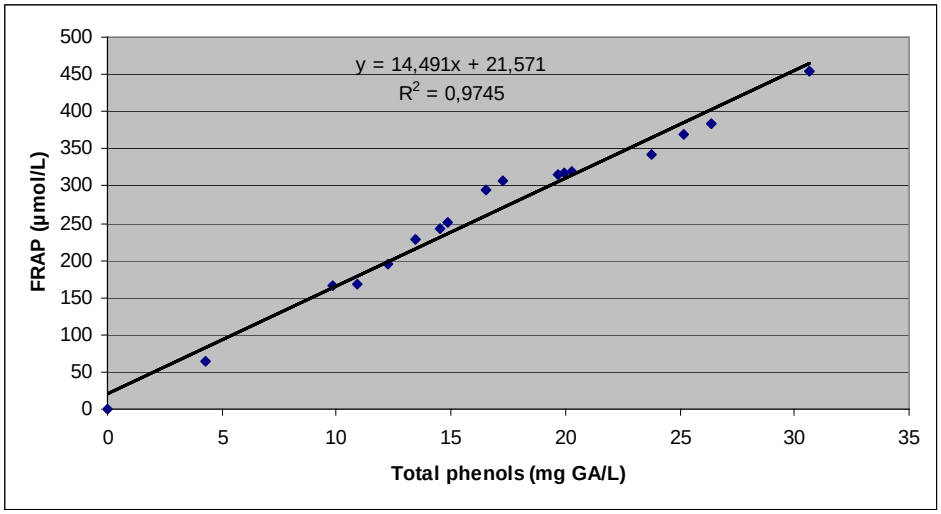


Table 2: The total phenolic content and related antioxidant capacity in the whole grain extract on 40°C.

Type of cereals and origin	Total phenolics (mg GA/L)	FRAP (µmol Fe^{II}/L)	PAC[a]
Buckwheat (Mrkonjic Grad, B&H)	30,65	453,8	2,51
Rye (Velika Kladusa, B&H)	26,34	383,33	2,47
Oats (Gnojnica-Lukavac, B&H)	25,19	368,59	2,48
Oats (Mrazovac-Buzim, B&H)	23,78	342,85	2,45
Oats (Velika Kladusa, B&H)	20,26	320	2,68
Barley (Velika Kladusa, B&H)	19,94	318,23	2,71
Barley (Mrazovac-Buzim, B&H)	19,68	316,09	2,72
Barley (Gnojnica-Lukavac, B&H)	17,26	307,14	3,02
Corn (Toplice, B&H)	16,56	295,52	3,03
Corn (Si Selo-Tuzla, B&H)	14,88	251,42	2,87
Corn (Srebrenik, B&H)	14,51	243,33	2,84
Wheat (Ljusine, B&H)	13,48	229,04	2,88
Wheat (Velika Kladusa, B&H)	12,24	194,28	2,69
Wheat (Bos.Krupa, B&H)	9,86	165	2,84
Brown rice (Italy)	10,89	168,57	2,63
White rice (Italy)	4,29	64,28	2,54

[a]PAC- Phenol antioxidant coefficient, calculated as ratio FRAP (µmol/L) / total phenolic (µmol GA/L)

Figure 2: Linear correlation between the amount of total phenols and antioxidant capacity (FRAP) in the extracts on 40°C.



The antioxidant coefficient (PAC) of the whole grain extracts calculated as ratio between FRAP (µmol Fe^{II}/L) and total phenolics (µmol GA/L), was used for quick comparison of antioxidant efficiency of total phenolics from different grain extracts. Calculated PAC values are relatively homogenized and they amount to 2,88-3,51 in extracts on 20ºC, and 2,51-3,02 in extracts on 40ºC. Higher values of total phenols and antioxidant capacity do not neccessarly make PCA higher. Results show that antioxidant activity of phenolic compounds amounts around 30% of total antioxidant capacity of tested cereal extracts. Considerable number of phenolic cereal compounds is soluble in organic and water-organic medium, therefore selection of different kind of solvent affect the values of tested parameters (Kim et al, 2006).

4. Conclusions

The results of conducted testing show the differences in values of total phenols and antioxidant capacity of different types of cereals. Increase in temperature influences the better extractibility of total

phenolic compounds and higher antioxidant capacity in all tested samples. The biggest antioxidant capacity, as well as the phenolic content was found in buckwheat. Therefore, this cereal would be the subject of further research. The results of the present study show significant lineral correlation between concentration of total phenolic compounds and antioxidant capacity of grain extracts. Therefore, it can be concluded that content of phenols in grain cereals is relevant indicator of reduction power. Further study would focus on the testing of antioxidant supstances from cereal (C vitamine, Selen), so as optimal extraction conditions of antioxidants (temperature, extraction time, solvent selection).

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