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THE EVALUATION OF ANTIOXIDANT ACTIVITY OF MILLING FRACTIONS OF SELECTED CEREALS GROWN IN THE YEAR 2010

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ABSTRACT

Cereals are good source of biologically active compounds that contribute to reducing the risk of coronary heart disease and also inhibit oxidation in human plasma. The aim of this study was to evaluate of antioxidant potential of four milling fractions of selected cereals grown in the year 2010. Methanol was used to extract the antioxidant compounds from cereals. Free radical scavenging activity of samples was measured using DPPH assay and reducing power was determined using FRAP assay. Secondary was evaluated of total phenolic and flavonoid content of cereal extracts. We found that flour fractions (break flour and reduction flour) showed the lower proportion of the total antioxidant potential than bran fractions (fine bran and coarse bran). Extract from barley had the highest values of antioxidant activity and phenolic content.

Keywords: cereals, milling fractions, phenolic, antioxidant activity

INTRODUCTION

Cereals are main foods in many countries, as human foods or as animal feeds **(Castro-Rubio et al. 2006)**. Epidemiological studies indicate that the consumption of whole-grain and whole-grain products is related to reduction in total mortality, coronary heart disease mortality, diabetes and cancer incidense **(Serpen et al. 2008)**. These beneficial effects are attributed to the bioactive factors in cereal grain such as non digestible carbohydrates and phytochemicals. The important part of phytochemicals with low molecular weight present in cereal grain is group of antioxidants such as tocopherols, lignans, flavonoids and phenolic acids. Antioxidants are defined as molecules that, at low concentration and specific assay conditions, can delay or prevent oxidation of an oxidizable substrate **(Vaher et al. 2010)**. Higher concentrations of these compounds are found in the outer layers of the kernel which constitute the bran **(Kim et al. 2006)**.

Cereal grains are rich in phenolic acids and saponins, while phytoestrogens and flavonoid are presented in small quantities **(Dordević et al. 2010)**. Studies have shown that dietary phenolics have high antioxidant activity, which may contribute to their health benefits. In cereals, the predominant phenolic acid is ferulic acid, representing up to 90 % of total polyphenols. Other phenolic acids including vanilic, syringic, chlorogenic, *p*-coumaric, *m*-coumaric and *OH*-cinnamic acid have also been reported in cereals **(Hosseinian & Mazza 2009)**. Total amount of polyphenols in cereals is highly variable both in whole grain and in bran and also depends on the cereal variety and milling procedure **(Adom et al. 2005)**.

The main objective of the present work was to evaluate of antioxidant potential of selected cereals by Free Radical Scavenging Activity (DPPH) and The Ferric Ion Reducing Antioxidant Power (FRAP), and its distribution into the dry milling fractions. In addition, the content of flavonoids and total phenolics was also determined, in order to refer of unutilized potential of naturally occurring antioxidants in cereals, which leaving in the form of bran during the production of flour.

MATERIAL AND METHODOLOGY

Chemicals were purchased from Sigma-Aldrich, St. Louis, MO and Mikrochem, SK. Cereals were grown in the year 2010 on a field nursery at Department of Environmental Protection and Organic Farming (DEPOF) Spišská Belá (SK) and on a field at Plant Production Research Institute (PPRI) Piešťany (SK). The used types and genotypes of cereals were: wheat (Torysa, PPRI), oat (Cacko, DEPOF), spelt wheat (Roquir, PPRI), triticale (Kandar, PPRI), rye (Dankovské nové, PPRI), barley (Ezer, DEPOF). Before the measurement samples were milled by laboratory mill (Brabender Quadrumat Senior) gaining four milling products: break flour (MF I.), reduction flour (MF II.), fine bran (MF III.), and coarse bran (MF IV.). 0.5 g of milling fractions was extracted with methanol for 24 hours. After centrifugation at 3000 g (Himac CT 6E, Hitachi Ltd., Japan,) for 20 min, the supernatant was evaporated at 40 °C and residue was solubilised in 1 mL of methanol.

Free Radical Scavenging Activity

Free radical scavenging activity of samples was measured using the 2,2-difenyl-1-picrylhydrazyl (DPPH) according to the procedures described by **Yen & Chen (1995)**. The extracts (25 μL) were reacted with 100 μL of DPPH solution (0.012 g DPPH in 100 mL methanol). Absorbance of the cereal extracts was determined using BioTek Microplate Reader (ELx800) at 550 nm. Free radical scavenging activity of the samples was expressed as mg Trolox equivalent antioxidant capacity per g of dry matter (mg TEAC g DM).

Reducing Power

Reducing power of samples was determined according to the procedure by **Oyanizu (1986)**. The mixture of cereal extract (20 μL), phosphate buffered saline (50 μL, pH 6.6) and 1% potassium ferricyanide (50 μL) was incubated at 50 °C for 20 min, then rapidly cooled, mixed with 50 μL of 10% trichloacetic acid, and centrifugated at 11 000 g (Eppendorf MiniSpin) for 10 min. 50 μL of the supernatant was mixed with 50 μl of distilled water and 10 μL of 0.1%

ferric chloride. The absorbance at 700 nm using BioTek Microplate Reader (ELx800) was detected. Reducing power was expressed as mg Trolox equivalent antioxidant capacity per g of dry matter (mg TEAC g DM).

Total Phenolic Content

Total phenolic content of cereal extracts was measured spectrophotometrically, using the modified Folin-Ciocalteu method as described by **Singleton et al. (1965)**. 0.1 mL of each cereal extract was mixed with 0.1 mL of the Folin-Ciocalteau reagent and 1 mL of 20% sodium carbonate, and centrifugated at 11 000 g (Eppendorf MiniSpin) for 10 min. 240 µL of the supernatant was used for measured the absorbance at 700 nm using BioTek Microplate Reader (ELx800). The total phenolics content was expressed as mg gallic acid equivalent (GAE) per g dry matter (DM).

Total Flavonoid Content

Total flavonoid content was determined using the modified method by **Quettier-Deleu et al. (2000)**. 0.1 mL of cereal extract was mixed with 20µL of 5% methanolic solution of aluminium chloride and centrifugated at 11 000 g (Eppendorf MiniSpin) for 10 min. 120 µL of supernatant was used for measured the absorbance at 405 nm on a BioTek Microplate Reader (ELx800). The total flavonoid content was expressed as mg quercetin equivalent (QE) per g dry matter (DM).

RESULTS AND DISCUSION

DPPH$^{\bullet}$ is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reductions capability of DPPH$^{\bullet}$ is determined by the decrease in its absorbance induced by antioxidant **(Liu & Yao, 2007)**. The scavenging effect of cereal extracts in flour milling fractions on DPPH radical decreased in this order: barley (29%) > spelt (22%) > oat (15%) > rye (13%) > triticale (12%) > wheat (9%). The scavenging effect of cereal extracts in bran milling fractions on DPPH radical decreased in this order: barley (20%) > triticale (18%) > wheat (17%) > oat (15%) > rye (15%) > spelt (14%). (Fig.1). These results indicated that all the extracts had a noticeable effect on scavenging free radical. The higher activities of all extracts were measured in bran (MF III. and MF IV.). Bran is a composite material made of several layers, such as pericarp, testa and aleurone **(Hemery et al., 2011)**. The main components of the fine bran (MF III.) are aleurone layer and the germ. In coarse bran (MF IV.) is dominant pericarp **(Schnürer, 1991)**. It is known that cereals bran are a rich source of fatty acids and several substances, such as tocopherol, vitamins, and phenolic compounds, possessing antioxidant properties **(Prisenžňáková et al., 2010)**.

The extract from barley had the strongest scavenging activity in flour milling fractions. From the literature it is known, that the barley is an excellent source of natural antioxidant either for food preservation (to inhibit lipid oxidation), or for disease prevention **(Fardet et al., 2008)**. **Liu & Yao (2007)** determined scavenging activity of barley seed extracts and found strong activity, which was dominant in 70 % acetone and shown similar activity to BHT at the amount of 200 µg. In bran fractions extract from rye and wheat showed the strongest activity. High activity was also determined in flour extracts of spelt and in bran extract of triticale. In a recent study **Hosseinian & Mazza (2009)** described triticale bran than potential new sources of antioxidant compounds.

Reducing power

For measurement of the reductive ability, the Fe^{3+} - Fe^{2+} transformation in the presence of cereal extracts was investigated. Reductive capabilities of cereal extracts shown Fig. 2. Increase in absorbance of the reaction mixture indicated the reducing power of the samples. Reducing power of flour milling fractions of cereal extracts exhibited the following order: barley (50%) > spelt (16%) > oat (13%) > rye (10%) > wheat (7%) > triticale (3%). Reducing power of bran milling fractions of cereal extracts exhibited the following order: barley (32%) > triticale (16%) > wheat (16%) > oat (14%) > rye (13%) > spelt (9%). Similar like DPPH assay, the higher activities of all extracts were measured in bran (MF III. and MF IV.).

The reducing capacity of a compound may serve a significant indicator of its potential antioxidant activity **(Liu & Yao, 2007)**. The reducing properties are generally associated with the presence of reductones **(Pin-Der, 1998)**. It is reported that the antioxidant action of reductones is based on the breaking of the free radical chain by donating a hydrogen atom, or reacting with certain precursors of peroxide to prevent peroxide formation. It is presented that the phenolic compounds in cereals may act in a similar fashion as reductones by donating electrons and reacting with free radicals to convert them to more stable products and terminating the free radical chain reaction **(Liu & Yao, 2007)**. The data presented here indicate that the marked reducing power of cereal extracts seem to be the result of their antioxidant activity.

Similar like DPPH assay, the extract from barley had the strongest reducing power in both years in all fractions**. Liu & Yao (2007)** determined reducing power of different solvent extracts of barley and found that 70 % methanol extract exhibit the highest activity. Zhao et al. (2008) measured reducing power of malting barley extract and confirmed strong activity of barley. The high activities were also determined in flour fractions of spelt, and in bran fractions of triticale and rye. **Zieliński et al. (2007)** reported that rye is an excellent raw material for healthy and tasty foods. The ray grain contains a large variety of substances, especially those that are biologically active and demonstrate antioxidant properties, which include free radical-scavengers, reducing agents, potential complexes of prooxidant metals and quenchers of the formation of singlet oxygen.

Cereal polyphenols

Phenolics are compounds with one or more aromatic ring and one or more hydroxyl groups **(Liu, 2003).** Structurally, phenolics in cereals can be subdivided into acids derived from either benzoic acid or cinnamic acid. Vanillic and salicylic acids are derivates of benzoic acid while ferulic acid, the dominant phenolic acid in cereals, and caffeic acid are derivates of cinnamic acid **(Abdel-Aal et al., 2001)**. Phenolic acids are predominantly found in the outer bran layer.

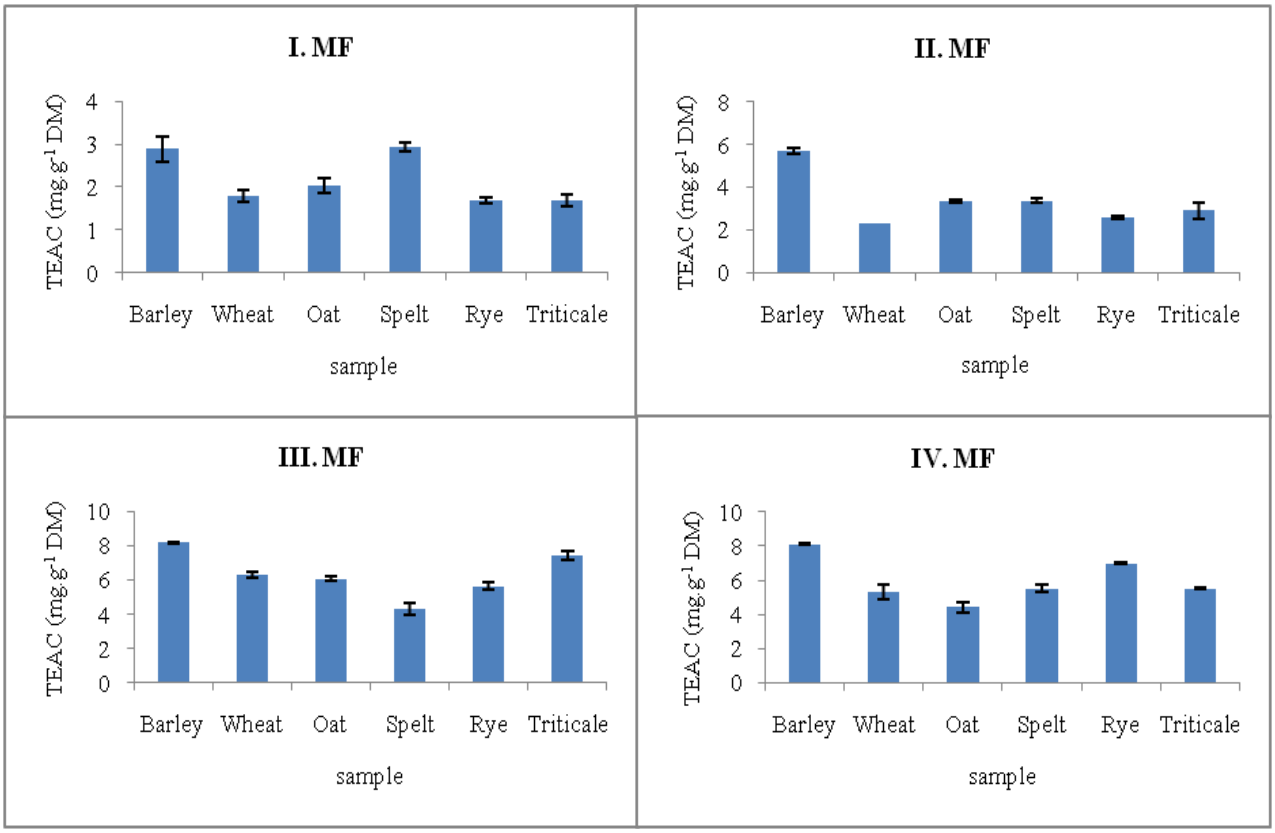

Fig. 1 Free radical scavenging activity of cereal extracts (in the year 2010) expressed as mg Trolox equivalent antioxidant capacity per g of dry matter (mg TEAC g DM), (I. MF – break flour, II. MF – reduction flour, III. MF – fine bran, IV. MF –coarse bran).

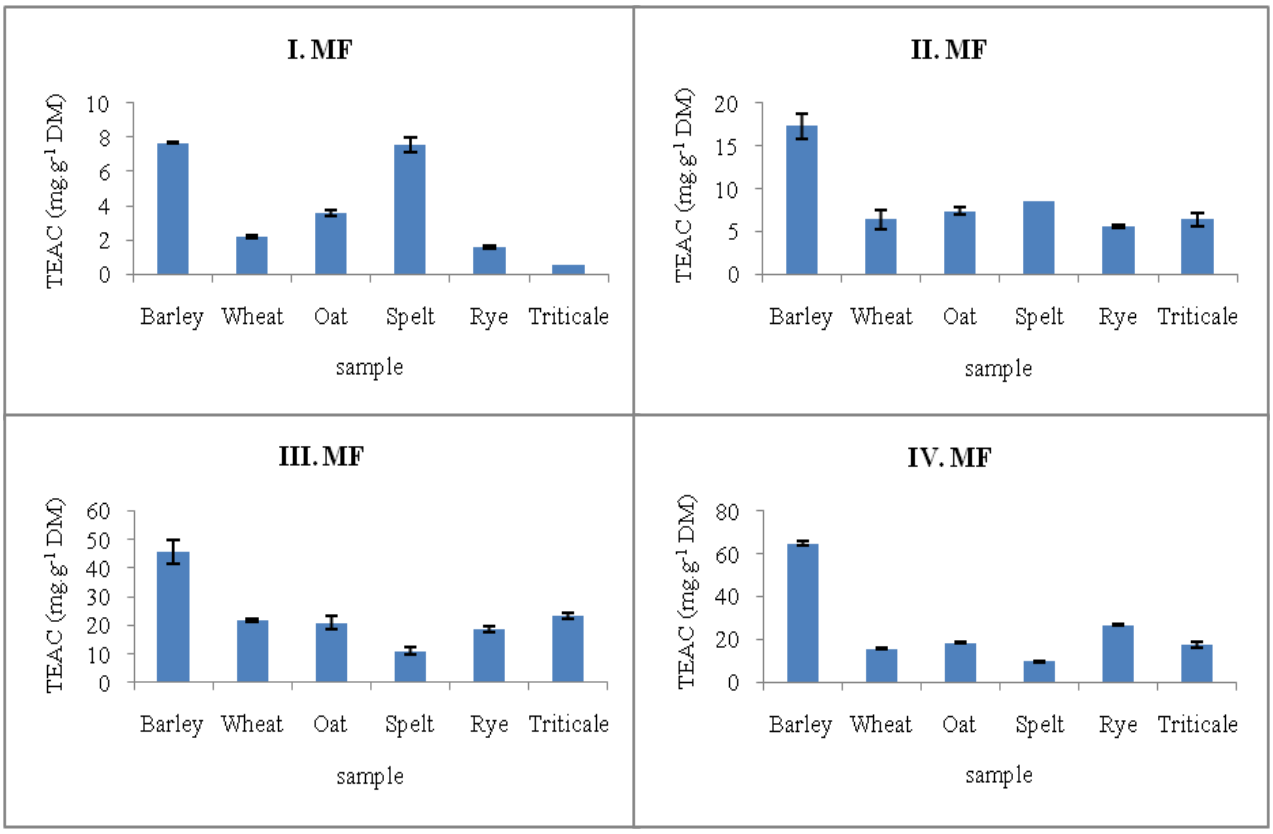

Fig. 2. Reducing power of cereal extracts (in the year 2010) expressed mg Trolox equivalent antioxidant capacity per g of dry matter (mg TEAC g DM), (I. MF – break flour, II. MF – reduction flour, III. MF – fine bran, IV. MF – coarse bran).

Flavonoids are one group of phenolics, which consists of two aromatic rings linked by 3 carbons that usually in an oxygenated heterocycle ring **(Liu, 2004)**. In cereals flavonoids are located mainly in the pericarp **(Dykes & Rooney, 2007)** in small quantities only **(Peterson, 2001)**. **Kim et al. (2006)** reported that wheat is a good source of phenolic acid and flavonoids. **King (1962)** isolated two related flavones glycosides from wheat germ. Three major flavones, apigenin, luteolin and tricin were identified in oat flour **(Peterson, 2001)**. In barley grains are dominant catechin and epicatechin **(Shahidi & Naczk, 2004)**.

The majority of phenolics in cereals are insoluble and bound by ester and ether linkages with polysaccharides, such as arabinoxylan and lignin, in the cell wall **(Liyana-Pathirana & Shahidi, 2006)**, while a smaller portion is soluble **(Stalikas, 2007)**. The bran layer is highly stratified not only in phenolic composition, but also in the degree of ester and ether bonds and the compounds to which the phenolics are cross-linked **(Verma et al., 2009)**. Several studies have shown that methanol is an effective solvent in extracting phenolics and other polar substances from cereals **(Ragaee et al., 2006)**. In this study, methanol extracts from cereals were used for the determination of phenolic (mg GAE/g) and flavonoid content (mg QE/g).

Total Phenolic Content

The total phenolic content was determined by the Folin-Ciocalteau assay. The results are presented in Tab. 1.

Table 1 The phenolic contents (mg GAE/g) of milling fractions from cereals grown in the year 2010.

Sample	Milling Fraction							
	I.		II.		III.		IV.	
Barley	31,4	± 1,2	62,1	± 0,7	161,0	± 4,1	291,7	± 3,5
Wheat	17,6	± 0,1	19,7	± 0,5	159,1	± 2,6	128,2	± 0,3
Oat	31,3	± 0,2	39,1	± 0,7	99,2	± 2,2	61,3	± 2,7
Spelt	40,4	± 0,7	36,4	± 0,3	66,4	± 1,0	151,0	± 3,7
Rye	17,6	± 0,3	30,3	± 0,6	95,9	± 1,6	159,5	± 12,6
Triticale	11,8	± 0,5	16,3	± 1,0	178,0	± 1,7	133,7	± 3,4

I. – bread flour, II. – reduction flour, III. – fine bran, IV. – coarse bran

From Tab. 1. it is evident that bran have higher content of total phenolic than flour. This is not surprising because it is well known that phenolic compounds are concentrated in the bran fractions of cereals that are removed during the milling of cereals into white flour **(Vaher et al., 2010; Kim et al., 2006)**. **Abdel-Aal et al., (2001)** invetigated the distribution of phenolic acids in wheat milling fractions. About 73 % of grain phenolic acids were found in the bran, but only 5 % in first and second milling fraction.

Total phenolic content of cereal extracts in flour milling fraction decreased in this order: barley > (26%) > spelt (26%) > oat (19%) > rye (15%) > wheat (8%) > triticale (6%). Total phenolic content of cereal extracts in bran milling fraction decreased in this order: barley > (21%) > wheat (20%) > triticale (17%) > rye (16%) > spelt (15%) > oat (11%). The results showed that phenolics were found in all cereal extracts, but bran fractions content higher values of phenolics. The sample of barley and spelt in flour fractions showed higher amounts of phenolics than extract from oat and wheat. In bran fractions were the highest amounts of phenolics determined in sample of barley, wheat and triticale.

A comparison of results from different studies can be difficult, because in our study cereals were milling into four milling fraction, while in other works, sample of cereals were milling into flour and bran.

Total Flavonoid Content

The total flavonoid content of cereal extracts is shown in Tab. 2.

Table 2 The flavonoid contents (mg QE/g) of milling fractions from cereals grown in the year 2010.

Sample	Milling Fraction							
	I.		II.		III.		IV.	
Barley	0,75	± 0,04	1,02	± 0,03	1,08	± 0,07	2,14	± 0,07
Wheat	0,12	± 0,01	0,16	± 0,01	0,93	± 0,15	3,07	± 0,06
Oat	0,64	± 0,04	0,84	± 0,05	2,60	± 0,02	1,23	± 0,05
Spelt	0,33	± 0,03	0,42	± 0,04	0,81	± 0,02	1,55	± 0,04
Rye	0,24	± 0,04	0,51	± 0,02	2,39	± 0,02	1,91	± 0,03
Triticale	0,11	± 0,02	0,18	± 0,02	1,01	± 0,02	1,24	± 0,04

I. – bread flour, II. – reduction flour, III. – fine bran, IV. – coarse bran

Higher concentration was found in bran (MF III. and MF IV.), but in smaller amounts than the total phenolic content. Bran flavonoids may be important for the miller because bran are introduced into flour during the milling process. Increasing amounts of bran will decrease the grade of the flour **(Feng et al., 1988)**.

In flour milling fractions of cereal extracts content of flavonoid decreased in this order: oat (31%) > barley (25%) > spelt (16%) > rye (15%) > triticale (6%) > wheat (6%). In bran milling fractions of cereal extracts content of flavonoid decreased in this order: wheat (20%) > rye (20%) > oat (19%) > barley (17%) > spelt (12%) > triticale (12%).

From literature is known that flavonoids are concentrated mainly in pericarp. Our results shown that flavonoids are presented also in flour fractions; this is very important information, because products from endosperm are basic in human nutrition. In flour milling fractions, high amount of total flavonoid showed extract of barley and oat. Oat is a source of many compounds that exhibit antioxidant activity, but is consumed in considerably lower quantities worldwide than wheat **(Peterson, 2001)**. In bran milling fractions high amount of total flavonoid was determined in extract of rye and wheat.

These results indicate that the flavonoids of cereals were mostly concentrated in the outer layer of grains. **Adom and Liu (2002)** found similar results for cereal grains including wheat and oat.

CONCLUSION

In this article, we prepared and evaluated milling fractions from selected cereals. Antioxidant activity was determined by DPPH and FRAP assay, and total phenolic and flavonoid content was also determined. We found that flour fractions (break flour and reduction flour) showed the lower proportion of the total antioxidant potential, which

was balanced in observed years. Bran fractions (fine bran and coarse bran) showed higher antioxidant activity, but 30 – 80 % of these fractions are unused in food industry, they are used mainly as animal feed. Extract from barley showed the highest values in all methods in observed years. It is evident, that bran fractions can be evaluated in the future and used for fortification of flours

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