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Antioxidant and free radical scavenging activities of whole wheat and milling fractions

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Abstract

The effects of milling on the phenolic content and antioxidant capacity of two wheat cultivars, namely CWAD (Canadian Western Amber Durum; *Triticum turgidum* L. var. durum) and CWRS (Canadian Western hard red spring; *Triticum aestivum* L.) were studied. The milling of wheat afforded several fractions, namely bran, flour, shorts and feed flour. In addition, semolina was the end-product of durum wheat milling. Among different milling fractions the bran had the highest phenolic content while the endosperm possessed the lowest amount and this was also reflected in free radical and reactive oxygen species (ROS) scavenging capacity, reducing power and iron (II) chelation capacity of different milling fractions in the two cultivars. This study demonstrated the importance of bran in the antioxidant activity of wheat, hence consumption of whole wheat grain may render beneficial health effects.

Keywords: *Triticum aestivum*; *T. turgidum*; Total phenolic content; Free radical scavenging; ROS scavenging; Reducing power; Iron (II) chelation; Milling fractions

1. Introduction

Plant foods such as grains, vegetables and fruits contain a wide variety of biologically active phytochemicals (Caragay, 1992). Plant-derived antioxidants may function as reducing agents, scavengers of free radicals and metal ion chelators, among others (Rice-Evans, Miller, & Paganga, 1996). The antioxidant activity of plasma has been shown to increase after consumption of foods high in antioxidants (Temple, 2000). Thus, phytochemicals may combat oxidative stress in the human body by maintaining a balance between oxidants and antioxidants (Temple, 2000). This is particularly important because under severe oxidative stress excessive formation of reactive oxygen species (ROS) and free radicals can damage biomolecules, such as DNA, proteins, lipids and carbohydrates, and lead to numerous disease conditions (Halliwell, 1996).

Cereals have been known to contain a high amount of hydroxycinnamic acid (HCA) derivatives that render potential health benefits (Andreasen, Landbo, Christensen, Hansen, & Meyer, 2001). Commercial processing of cereals may lead to products with low value fractions such as hulls and polish waste. In general, hulls are removed prior to food production. However, these low value fractions may serve as potential sources of natural antioxidants at relatively high concentrations (Bryngelsson, Dimberg, & Kamal-Eldin, 2002). In oats antioxidant compounds are mostly concentrated in the bran as compared to that in the endosperm as shown by in vitro assays (Peterson, 2001; Peterson, Emmons, & Hibbs, 2001). Emmons, Peterson, and Paul (1999) demonstrated higher antioxidant activity and total phenolic content (TPC) in three oat pearling fractions containing different levels of bran layers compared to those of the flour extracts. According to Yu, Haley, Perret, and Harris (2002a) bran extracts of three different wheat varieties exhibited significant antioxidant properties against free radical scavenging and metal

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ion chelation. Moreover, whole grains of three hard wheat varieties exhibited antioxidant activity against lipid peroxidation in a fish oil model system (Yu et al., 2002b). The 'Akron' variety of wheat was highly effective in scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and chelating Fe(II). Zhou and Yu (2004) reported the effects of growing conditions employed on antioxidant activity of a wheat variety grown in different locations. These authors reported that TPC, scavenging of DPPH radical and chelation of Fe(II) were significantly influenced by agronomic practices and environmental conditions. The antioxidant properties of whole grains, bran and aleurone layer of a Swiss red wheat variety was studied using free radical scavenging and metal ion chelation capacity (Zhou, Laux, & Yu, 2004). Thus, aleurone, bran and grains differed significantly in their antioxidant potential, TPC and phenolic acid composition. Moreover, the aleurone layer exhibited the highest antioxidant activity, TPC and content of phenolic acids (Zhou et al., 2004). Ferulic acid was reported to be the predominant phenolic acid accounting for approximately 57–77% of total phenolic acids present in wheat on a dry weight basis. Ferulic acid content was positively correlated with scavenging of free radicals and TPC and hence may be used as a potential marker of wheat antioxidants (Zhou et al., 2004). Plant phenolic compounds including phenolic acids, flavonoids and anthocyanins, among others, have also been recognized as conferring stability against autoxidation of vegetable oils (van Ruth, Shaker, & Morrissey, 2001). There is much interest in the use of crude phenolic extracts from fruits, herbs, vegetables, cereals and other plant materials in the food and supplement industry because they have been shown to retard oxidative degradation processes, especially those of lipids thereby improving the quality and nutritional value of food (Kähkönen et al., 1999). The effects of milling on the antioxidant potential of whole grains and their milling fractions, namely bran, flour, feed flour and shorts of two wheat cultivars under in vitro conditions were determined.

2. Materials and methods

2.1. Materials

The compounds 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis[3-ethylbenz-thiazoline-6-sulphonate (ABTS), 2,2′-azobis-(2-methylpropionamidine) dihydrochloride (AAPH) 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin–Ciocalteu's phenol reagent, ferulic acid, hypoxanthine, xanthine oxidase, nitro blue tetrazolium, sodium carbonate, monobasic potassium phosphate, dibasic potassium phosphate, hydrogen peroxide, ethylenediaminetetraacetic acid (EDTA), L-ascorbic acid, 2-deoxyribose, ferric chloride, ferrozine and ferrous chloride were purchased from Sigma–Aldrich Chemical Co. (Oakville, ON). All other solvents were purchased from Fisher Scientific (Nepean, ON) and were of ACS grade or better.

Samples of the two prominent wheat classes grown in western Canada, CWAD (Canadian Western Amber Durum; *Triticum turgidum* L. var. durum) and CWRS (Canadian Western red spring; *Triticum aestivum* L.), and their milling fractions, namely bran, flour, shorts and feed flour, (crop year 2002 and crop year 2001, respectively), were obtained from Canadian Grain Commission, Winnipeg, MN.

2.2. Sample preparation

Whole grains and their milling fractions, when necessary, were further ground in a coffee grinder (Model CBG5 series, Black and Decker Canada Inc., Brockville, ON) in order to pass through a mesh size 16 sieve (Tylor Test Sieve, Mentor, OH). Wheat flour was used as such for the extraction of crude phenolics. All samples were defatted by blending the ground material with hexane (1:5, w/v, 5 min, 3×) in a Waring blender (Model 33BL73, Waring Products Division, Dynamics Corp. of America, New Hartford, CT) at ambient temperature. Defatted wheat samples were air dried for 12 h and stored in vacuum packaged polyethylene pouches at −20 °C for further analysis.

2.3. Extraction of crude phenolics of wheat

The crude phenolic compounds present in whole grains and their milling fractions were extracted into 80% aqueous ethanol (1:10, w/v) at 4 °C for 16 h. The resulting slurries were centrifuged at 4000*g* for 5 min and the supernatants were collected. The residues were re-extracted (1:5, w/v; 80% ethanol) under the same conditions and supernatants from both extractions were combined. The solvent was removed under vacuum at 40 °C and the resulting concentrated slurries were lyophilized for 72 h at −47 °C and 69×10^{-3} mbar. Yields of the crude phenolic extracts were reported as percentage of defatted materials.

2.4. Determination of total phenolics content (TPC)

The content of total phenolics was determined according to a modified version of the procedure described by Singleton and Rossi (1965). Extracts were dissolved in methanol to obtain a 5 mg/mL concentration solution. Folin–Ciocalteu's reagent (0.5 mL) was added to a centrifuge tube (50 mL) containing 0.5 mL of the extract. Contents were mixed and 1 mL of saturated sodium carbonate solution was added to each tube, followed by adjusting the volume to 10 mL with distilled water. The contents in the tubes were thoroughly mixed by vortexing. Tubes were allowed to stand at ambient temperature for 45 min until the characteristic blue colour developed; centrifugation was then carried out for 5 min at 4000*g* (ICE Centra M5, International Equipment Co., Needham Heights, MA). Absorbance of the clear supernatants was measured at 725 nm using a diode array spectrophotometer (Model 8452A,

Agilent Technologies Canada Inc., Mississauga, ON). The content of total phenolics in each extract was calculated by employing a standard curve prepared using ferulic acid and expressed as micrograms of ferulic acid equivalents (FAE) per gram of defatted material.

2.5. Measurement of total antioxidant capacity (TAC)

Total antioxidant activity was determined according to the Trolox equivalent antioxidant capacity (TEAC) assay described by van den Berg, Haenen, van den Berg, and Bast (1999) with slight modifications. The extracts and reagents were prepared in a 0.1 M phosphate buffer (pH 7.4) containing 0.15 M sodium chloride (PBS). A solution of ABTS radical anion ($ABTS^{\bullet-}$) was prepared by mixing 2.5 mM AAPH with 2.0 mM solution of $ABTS^{2-}$ at a 1:1 (v/v) ratio, and heating at 60 °C for 12 min. The absorbance of the freshly prepared radical solution at 734 nm was about 0.4. The radical solution protected from light was stored at room temperature. A standard curve was prepared using different concentrations of Trolox. Thus, the reduction in the absorbance (ΔA) of the $ABTS^{\bullet-}$ solution (1960 μL) at different concentrations of Trolox (40 μL) over a period of 6 min was measured and plotted. The TEAC values of wheat extracts (5 mg/mL) were determined in the same manner and expressed as μmol Trolox equivalents/g of defatted material. A blank was used for each measurement that corresponded to a decrease in absorbance without any compound or extract added. The TEAC of an unknown compound represents the concentration of a Trolox solution that has the same antioxidant capcity as the compound of interest.

TEAC values were determined as follows:

$$\Delta A_{\text{Trolox}} = (A_{t=0\ \text{min Trolox}} - A_{t=6\ \text{min Trolox}}) - \Delta A_{\text{Radical(0–6 min)}},$$

$$\Delta A_{\text{Trolox}} = m \times [\text{Trolox}],$$

$$\text{TEAC}_{\text{Extract}} = (\Delta A_{\text{Extract}}/m) \times d,$$

where ΔA is the reduction of absorbance, A the absorbance at a given time, m the slope of the standard curve, [Trolox] the concentration of Trolox and d is the dilution factor.

2.6. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay

The method described by Kitts, Wijewickreme, and Hu (2000) was used with slight modifications in order to assess the DPPH radical-scavenging capacity of wheat extracts. A 0.075 mM (final concentration) DPPH solution in ethanol was mixed with wheat extracts and vortexed thoroughly. The absorbance of the mixtures at ambient temperature was recorded for 60 min at 10 min intervals. The absorbance of the remaining DPPH radicals was measured at 519 nm using a diode array spectrophotometer (Model 8452A, Agilent Technologies Canada Inc., Mississauga, ON). The scavenging of DPPH was calculated according to the following equation:

$$\%\ \text{scavenging} = \{(\text{Abs}_{\text{contol}} - \text{Abs}_{\text{sample}})/\text{Abs}_{\text{control}}\} \times 100,$$

where $\text{Abs}_{\text{control}}$ = absorbance of DPPH radical + methanol; $\text{Abs}_{\text{sample}}$ = absorbance of DPPH radical + wheat extract/standard.

The scavenging capacity was expressed as μmol DPPH radical scavenged/g of defatted material.

2.7. Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging assay was carried out following the procedure of Ruch, Cheng, and Klauring (1989). Wheat samples were dissolved in 3.4 mL of 0.1 M phosphate buffer (pH 7.4) and mixed with 0.6 mL of a 43 mM solution of hydrogen peroxide prepared in the same buffer. The absorbance of the reaction mixture was recorded over a 40-min period at 10 min intervals at 234 nm. A blank sample devoid of hydrogen peroxide was used for background subtraction. The concentration of hydrogen peroxide in the assay medium was determined using a standard curve and hydrogen peroxide scavenging activity of samples was calculated using the following equation.

Hydrogen Peroxide Scavenging Capacity (%) = 100 − [(hydrogen peroxide concentration of medium containing the extract)/(hydrogen peroxide concentration of the control medium)] × 100.

Antioxidant activity was expressed as μmol hydrogen peroxide scavenged/g of defatted material.

2.8. Superoxide radical scavenging activity

The superoxide radical was generated with an enzymatic reaction according to a modified version of the method explained by Nishikimi, Rao, and Yagi (1972). The reaction mixture contained 1 mL of each of 3 mM hypoxanthine, xanthine oxidase (100 mIU), 12 mM diethylenetriaminepentaacetic acid, 178 mM nitro blue tetrazolium and the sample. The absorbance of the medium was read at 560 nm over a 60 min period at 10 min intervals. The absorbance values were corrected by subtracting 0 min readings from those obtained subsequently. Superoxide radical scavenging activity (at 10 min) of additives was calculated using the following equation.

Superoxide Radical Scavenging Capacity (%) = 100 − [(absorbance of medium containing the additive of concern)/(absorbance of the control medium)] × 100.

Antioxidant activity of wheat samples was expressed as nmol superoxide radical anion scavenged/g of defatted material.

2.9. Reducing power of wheat extracts

The reducing power of wheat samples was determined following the method of Oyaizu (1986) with some modifications. The assay medium contained 2.5 mL of sample in a 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1%

potassium ferricyanide. After incubation at 50 °C for 20 min, 2.5 mL of 10% trichloroacetic acid were added to the mixture followed by centrifugation at 1750*g* for 10 min. One millilitre of the supernatant was mixed with 2.5 mL HPLC-grade water and 0.5 mL of 0.1% ferric chloride, and the absorbance of the resultant solution was read at 700 nm. A standard curve was prepared using various concentrations of ascorbic acid and the reducing power was expressed as μmol ascorbic acid equivalents/g of defatted material.

2.10. Hydroxyl radical scavenging capacity of wheat extracts

The effect of hydroxyl radical was assayed using the deoxyribose method as described by Halliwell, Gutteridge, and Grootveld (1987). One millilitre of final reaction mixture contained 500 μL of extracts and 100 μL of each of 100 μM $FeCl_3$, 100 μM EDTA, 20 mM H_2O_2, 100 μM L-ascorbic acid and 30 mM deoxyribose in 0.2 M phosphate buffer (pH 7.4). The reaction mixture was incubated at 37 °C for 1 h followed by heating in a boiling water bath for 15 min after addition of 1 mL of trichloroacetic acid (2.8%, w/v) and 1 mL of a 1% (w/v) solution of 2-thiobarbituric acid. The absorbance of the solution was measured at 532 nm against a phosphate buffer blank. Antioxidant capacity was expressed as μmol hydroxyl radical scavenged/g of defatted material.

2.11. Measurement of iron (II) chelating activity of wheat extracts

The Fe(II) chelating activity of wheat extracts was measured as reported by Carter (1971). The reaction was performed in an aqueous medium. The wheat extracts (3 or 6 mg/mL, 2.0 mL) were mixed thoroughly with 2 mM $FeCl_2$ (0.2 mL) and 5 mM ferrozine (0.4 mL). The mixtures were left at room temperature for 10 min. The absorbance of the resultant solution was read at 562 nm. The Fe(II) chelating activity of wheat extracts was calculated as follows:

$$\text{Iron (II) chelating activity (\%)} = \{1 - (\text{absorbance of sample at 562 nm}/\text{absorbance of control at 562 nm})\} \times 100.$$

The iron (II) chelating capacity of samples was expressed as μg EDTA equivalents/g of defatted material using a standard curve prepared with EDTA.

2.12. Statistical analysis

All analyses were performed in triplicate and data reported as mean ± standard deviation, unless otherwise stated. Analyses of variance were performed using General Linear Model of Minitab Release 14 Xtra for Windows (Minitab Inc., State College, PA). Significant differences ($P < 0.05$) among means were determined using the Tukey's multiple range test at a fixed level of $\alpha = 0.05$.

3. Results and discussion

3.1. Total phenolics content (TPC) and total antioxidant capacity (TAC) of wheat milling fractions

The yield of crude extracts, total phenolics content (TPC) and total antioxidant capacity (TAC) of whole wheat grains and milling fractions of cultivars CWAD and CWRS are shown in Table 1. The cultivar CWAD is mainly grown for production of semolina and pasta while CWRS cultivar is a common bread wheat. The amount of extractable matter varied significantly ($p < 0.05$) among whole grains and their milling fractions and hence produced varying extraction yields. The differences were also significant ($p < 0.05$) between the two cultivars examined. The TPC of different fractions ranged from 140 to 2279 μg FAE/g of defatted wheat and 216 to 3437 μg FAE/g of defatted wheat, respectively, for CWAD and CWRS. In both cultivars the bran fraction possessed the highest TPC. The order of TPC was bran > shorts > feed flour > whole grain > flour, for CWAD and CWRS. With CWAD there was an additional fraction, semolina, that possessed the lowest TPC. The TAC expressed as μM Trolox equivalents (TE) demonstrated a positive correlation with the TPC values. Both TPC and TAC were higher in the outermost layers of the wheat grain. Hence, bran was significantly higher in TPC and TAC compared to those of the endosperm that form the flour fraction.

Table 1
Extraction yield (%, w/w), total phenolic content (TPC; μg FAE/g defatted material) and total antioxidant capacity (TAC; μmol TE/g defatted material) of milling fractions of two wheat cultivars, CWAD and CWRS

Milling fraction	Crude yield		TPC		TAC	
	CWAD	CWRS	CWAD	CWRS	CWAD	CWRS
Whole grain	6.2 ± 0.4^{b}	5.4 ± 0.4^{a}	769 ± 41^{c}	1291 ± 31^{b}	4.24 ± 0.03^{c}	4.99 ± 0.06^{b}
Bran	10.6 ± 0.7^{de}	10.2 ± 0.8^{c}	2279 ± 61^{f}	3437 ± 86^{d}	10.32 ± 0.08^{f}	12.79 ± 0.09^{e}
Flour	4.2 ± 0.9^{c}	4.0 ± 0.5^{a}	210 ± 11^{b}	216 ± 16^{a}	2.24 ± 0.04^{b}	2.35 ± 0.02^{a}
Shorts	10.0 ± 1.1^{e}	9.8 ± 0.7^{c}	1920 ± 22^{e}	3146 ± 97^{c}	8.8 ± 0.06^{e}	11.53 ± 0.05^{d}
Feed flour	9.0 ± 0.8^{d}	8.1 ± 0.3^{b}	1404 ± 64^{d}	2033 ± 83^{b}	6.78 ± 0.03^{d}	8.96 ± 0.04^{c}
Semolina	5.2 ± 0.6^{a}	–	140 ± 13^{a}	–	1.69 ± 0.02^{a}	–

Values are means of three determinations ± standard deviation.
Values in each column with the same superscript are not different ($p > 0.05$).
Abbreviations: CWAD, Canadian Western Amber Durum; CWRS, Canadian Western Red Spring.

Semolina is also a product derived from endosperm. Semolina is obtained from durum wheat, for instance the cultivar CWAD, examined in this study. Semolina possessed the lowest TPC and TAC, with respect to the fractions examined with the CWAD cultivar. The higher TPC and TAC of shorts and feed flour compared to that of flour and semolina can be attributed to the presence of various proportions of bran and germ in them. In whole grains, on the other hand, the antioxidants get diluted due to the endosperm. The Trolox equivalent antioxidant capacity (TEAC) values, have been used to rank the antioxidant activity of unknown mixtures (van den Berg et al., 1999). Thus, the TEAC of a compound determines its antioxidant activity relative to that of Trolox. Another study on milling and sieving to obtain bran-rich and starch-rich fractions of oat revealed a higher TPC and antioxidant activity in the bran-rich fraction than those in the starch-rich fraction (Gray et al., 2000).

3.2. Scavenging of free radicals and reactive oxygen species (ROS) by wheat phenolics

The capacity of wheat samples to scavenge the stable DPPH radical is shown in Table 2 while Table 3 summarizes the results for quenching of biologically important reactive oxygen species (ROS) such as hydroxyl radical ($HO^{\cdot}$) and superoxide radical anion ($O_2^{\cdot-}$) as well as hydrogen peroxide (H_2O_2). The ability to scavenge DPPH radicals by wheat fractions was in the order of bran > shorts > feed flour > whole grain > flour, for both wheat cultivars. In addition, the CWAD semolina fraction possessed the lowest scavenging capacity for these ROS.

The wheat samples examined demonstrated significant scavenging capacity against $HO^{\cdot}$ as measured by the deoxyribose assay (Table 3). Superoxide anion radical scavenging capacity of wheat fractions was measured using the xanthine–xanthine oxidase system and the results were reported as nmol $O_2^{\cdot-}$ scavenged/g of defatted material (Table 3). While the efficiency of wheat fractions in scavenging $HO^{\cdot}$ and H_2O_2 was excellent, they were not effective in scavenging $O_2^{\cdot-}$. The samples of wheat especially those of bran, shorts, feed flour and whole grain demonstrated very high activity against scavenging of $HO^{\cdot}$ and H_2O_2 (Table 3). With respect to CWAD, the H_2O_2 scavenging varied from 263 ± 6 to 728 ± 12 μmol/g of defatted wheat. The corresponding values for CWRS were 264 ± 4 to 597 ± 16 μmol/g of defatted wheat. The flour and semolina fractions demonstrated the lowest scavenging capacity against H_2O_2. The ability to scavenge free radicals and ROS of wheat fractions was assessed using several in vitro antioxidant models. The ethanolic extracts of wheat could scavenge free radicals and ROS at the stage of initiation of a chain reaction thereby terminating the reaction. The results indicated wheat antioxidants, particularly those present in the bran, were efficient free radical scavengers.

Table 2
DPPH radical scavenging capacity (μmol/g of defatted material) of whole grains and their milling fractions of two wheat cultivars, namely CWAD and CWRS

Milling fraction	CWAD	CWRS
Whole grain	210 ± 1.4^{c}	218 ± 1.5^{b}
Bran	415 ± 1.9^{e}	429 ± 1.8^{d}
Flour	96 ± 1.6^{a}	100 ± 2.4^{a}
Shorts	342 ± 3.0^{d}	406 ± 3.1^{c}
Feed flour	318 ± 2.0^{d}	338 ± 3.5^{c}
Semolina	114 ± 1.3^{b}	–

Values are means of three determinations ± standard deviation.
Values in each column with the same superscript are not different ($p > 0.05$).
Abbreviations: CWAD, Canadian Western Amber Durum; CWRS, Canadian Western Red Spring.

Table 3
ROS scavenging capacity (hydroxyl radical; μmol/g defatted material, superoxide radical anion; nmol/g defatted wheat, hydrogen peroxide; μmol/g defatted wheat) of whole grains and milling fractions of two wheat cultivars, CWAD and CWRS

Milling fraction	CWAD	WRS
Hydroxyl radical scavenging		
Whole grain	24.1 ± 0.2^{d}	22.5 ± 0.4^{b}
Bran	47.8 ± 1.2^{e}	48.1 ± 0.5^{c}
Flour	21.1 ± 0.8^{b}	18.3 ± 0.3^{a}
Shorts	50.9 ± 2.0^{e}	43.8 ± 0.8^{c}
Feed flour	34.4 ± 0.7^{c}	33.3 ± 0.9^{b}
Semolina	13.4 ± 0.4^{a}	–
Superoxide anion radical scavenging		
Whole grain	242 ± 3^{c}	249 ± 2^{d}
Bran	439 ± 6^{e}	497 ± 2^{e}
Flour	149 ± 2^{b}	174 ± 2^{a}
Shorts	444 ± 7^{d}	421 ± 4^{c}
Feed flour	332 ± 2^{e}	319 ± 1^{b}
Semolina	87 ± 2^{a}	–
Hydrogen peroxide scavenging		
Whole grain	333 ± 7^{c}	324 ± 3^{b}
Bran	633 ± 7^{e}	621 ± 0^{c}
Flour	301 ± 4^{b}	264 ± 4^{a}
Shorts	712 ± 18^{cd}	597 ± 0^{c}
Feed flour	728 ± 12^{d}	493 ± 0^{c}
Semolina	263 ± 6^{a}	–

Values are means of three determinations ± standard deviation.
Values in each column with the same superscript are not different ($p > 0.05$).
Abbreviations: CWAD, Canadian Western Amber Durum; CWRS, Canadian Western Red Spring.

3.3. Reducing power of wheat phenolics

Table 4 shows the reducing power of phenolic compounds of whole grains and milling fractions of two wheat cultivars, CWAD and CWRS, as determined by the potassium ferricyanide reduction method. The bran fraction of CWRS possessed a strong (1068 μmol/g of defatted wheat ascorbic acid equivalents) reducing power. The reducing power of different milling fractions were significantly ($p < 0.05$) different from one another. The flour and semolina had the weakest reducing power among all fractions

Table 4
Reducing power (μmol ascorbic acid equivalents/g defatted material) of whole grains and milling fractions of two wheat cultivars, CWAD and CWRS

Milling fraction	CWAD	CWRS
Whole grain	367 ± 18^{b}	405 ± 14^{b}
Bran	949 ± 23^{e}	1088 ± 24^{e}
Flour	99 ± 4^{a}	131 ± 12^{a}
Shorts	811 ± 17^{d}	1009 ± 23^{d}
Feed flour	620 ± 26^{c}	777 ± 26^{c}
Semolina	100 ± 9^{a}	–

Values are means of three determinations ± standard deviation.
Values in each column with the same superscript are not different ($p > 0.05$).
Abbreviations: CWAD, Canadian Western Amber Durum; CWRS, Canadian Western Red Spring.

Table 5
Iron (II) chelation capacity (μg EDTA equivalents/g defatted material) of whole grains and their milling fractions of two wheat cultivars, CWAD and CWRS

Milling fraction	CWAD	CWRS
Whole grain	859 ± 36^{c}	894 ± 17^{b}
Bran	1276 ± 58^{f}	1316 ± 53^{d}
Flour	595 ± 12^{a}	616 ± 13^{a}
Shorts	1121 ± 31^{e}	1102 ± 49^{c}
Feed flour	903 ± 39^{d}	744 ± 34^{b}
Semolina	679 ± 18^{b}	–

Values are means of three determinations ± standard deviation.
Values in each column with the same superscript are not different ($p > 0.05$).
Abbreviations: CWAD, Canadian Western Amber Durum; CWRS, Canadian Western Red Spring.

examined. The reducing power of the bran layers, in general, was better than that of the endosperm. Hence, the bran alone, shorts and feed flour exhibited the highest reducing power. The reducing power of samples of the CWRS cultivar was significantly higher than that of CWAD. Shimada, Fijikawa, Yahara, and Nakamura (1992) reported that the reducing power of a compound may be attributed to its hydrogen-donating ability. These authors reported that ascorbic acid was a strong reductone that could readily donate a hydrogen atom to a free radical, thus terminating free radical reactions.

Duh (1998) also stated that reductones are efficient reducing agents and their efficiency is attributed to their hydrogen-donating ability. The wheat extracts examined in this study demonstrated good reducing capacity thereby acting as efficient reductones. The results on reducing power demonstrate the electron donor properties of wheat extracts thereby neutralizing free radicals by forming stable products. The outcome of the reducing reaction is to terminate the radical chain reactions that may otherwise be very damaging (Yen & Chen, 1995).

3.4. Iron (II)-chelation capacity of wheat phenolics

Iron (II)-chelation may render important antioxidative effects by retarding metal-catalyzed oxidation (Kehrer, 2000). The effective iron (II) chelators may also afford protection against oxidative damage by removing iron (II) that may otherwise participate in HO$^{\bullet}$-generating Fenton type reactions. Minimizing iron (II) may afford protection against oxidative damage by inhibiting production of ROS and lipid peroxidation. The iron (II)-chelating capacity of both whole wheat grains and their milling fractions was determined by measuring the iron–ferrozine complex and results are summarized in Table 5. In the iron (II)-chelation assay, bran demonstrated superior chelating properties over the other fractions. Flour and semolina had the lowest iron (II)-chelation capacity. Results indicated the presence of iron (II) chelating agents such as phenolics in association with bran layers. The ethanolic extracts of flour possessed greater iron (II)-chelation capacity than the other fractions (results not shown). However, iron chelation capacities were then expressed on the weight basis of the crude extract. In this paper, on the other hand, iron (II)-chelation capacity is expressed on the basis of defatted wheat and hence the existing difference in the observed trend. The extraction yields of various milling fractions were different thus causing the above deviation.

4. Conclusions

Asymmetrical distribution of antioxidative components in the wheat grain was prominent. The concentration of bioactive constituents was greater in the external layers; thus the bran fraction alone demonstrated a higher antioxidant activity than that of other milling fractions. The shorts and feed flour fractions are also known to contain bran and germ fractions in different proportions. Thus, uniformity cannot be expected with the shorts and feed flour. The antioxidant activity may vary depending on the actual composition of these fractions.

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