Phenolic Content and Antioxidant Activity of Pearled Wheat and Roller-Milled Fractions

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ABSTRACT

Wheat contains phenolic compounds concentrated mainly in bran tissues. This study examined the distribution of phenolics and antioxidant activities in wheat fractions derived from pearling and roller milling. Debranning (pearling) of wheat before milling is becoming increasingly accepted by the milling industry as a means of improving wheat roller-milling performance, making it of interest to determine the concentration of ferulic acid at various degrees of pearling. Eight cultivar samples were used, including five genotypes representing four commercial Canadian wheat classes with different intrinsic qualities. Wheat was pearled incrementally to obtain five fractions, each representing an amount of product equivalent to 5% of initial sample weight. Wheat was also roller milled without debranning. Total phenolic content of fractions was determined using the modified Folin-Ciocalteau method for all pearling fractions, and for bran, shorts, bran flour, and first middlings flour from roller milling. Antioxidant activity was determined on phenolic extracts by a method involving the use of the free radical 2,2-diphenyl-l-picrylhydrazyl (DPPH). Total phenolics were concentrated in fractions from the first and second pearlings (>4,000 mg/kg). Wheat fractions from the third and fourth pearlings still contained high phenolic content (>3,000 mg/kg). A similar trend was observed in antioxidant activity of the milled fractions with ≈4,000 mg/kg in bran and shorts, ≈3,000 mg/kg in bran flour, and <1,000 mg/kg in first middlings flour. Total phenolic content and antioxidant activity were highly correlated (R^2 = 0.94). There were no significant differences between red and white wheat samples. A strong influence of environment (growing location) was indicated. Pearling represents an effective technique to obtain wheat bran fractions enriched in phenolics and antioxidants, thereby maximizing health benefits associated with wheat-based products.

Phenolic compounds (phenolic acids, simple flavonoids, and proanthocyanidins) form a major group of phytochemicals found in plants. The interest in phenolics has been spurred by recent reports of their antioxidant activity. It is of some interest that wheat and other cereals contain phenolic compounds (Jende-Strid 1985; Onyeneho and Hettiarachchy 1992; Beta et al 1999; Andreasen et al 2000). These phenolics are chiefly present in the outer bran layers of the kernel and this has led to the use of tests to determine ferulic acid (a major phenolic acid) to measure the degree of bran carryover in flour milling (Pussayanawin and Wetzel 1987). Ferulic acids may be, in part, responsible for producing insoluble dietary fiber owing to their role in cross-linking arabinoxylans (Faulds et al 1997; Nishizawa et al 1998; Renger and Steinhart 2000). As part of the human diet, phenolics may contribute to the beneficial effects derived from consumption of cereal bran.

Phenolic content varies significantly among wheat cultivars (McCallum and Walker 1990; Hatcher and Kruger 1997). Significant variation in phenolic content also occurs between milling streams (McCallum and Walker 1990; Hatcher and Kruger 1997). Gao et al (2002) reported a range of 1,000–3,000 mg/kg of phenolic content in flour and bran. Work on wheat phenolics has largely focused on the effects exerted on wheat flour quality, particularly involvement in pigmentation of both flour and bread (McCallum and Walker 1990). However, with the increasing popularity of functional foods, it has become important to focus on cereal fractions with potential health benefits. In addition to its value as dietary fiber, wheat bran can be a valuable source of phenolic compounds. Fractionation by traditional roller milling of wheat separates bran layers enriched in phenolic compounds. The distribution of phenolic compounds in mill streams may have important implications in end-use applications and in generating health benefits as functional foods. Debranning (preprocessing, pearling) of wheat before roller milling is becoming increasingly accepted by wheat millers as a means to improve milling performance. The by-products of debranning hold great promise as functional food ingredients that differ from those of traditionally available mill fractions (Dexter and Wood 1996). The objectives of the current study were threefold: 1) to determine the phenolic content of fractions derived from sequential pearling of wheat and from roller milling of unpearled wheat; 2) to assess the antioxidant activity of phenolic extracts of wheat fractions against free radicals; and 3) to determine whether growing location has any effect on phenolic content and antioxidant activity.

MATERIALS AND METHODS

Eight Canadian wheat samples were used, including six genotypes representing four commercial classes of diverse technological quality. AC Barrie (Canada Western Red Spring), AC Superb (Canada Western Red Spring), AC Crystal (Canada Prairie Spring Red), AC Vista (Canada Prairie Spring White), AC Corinne (Canada Western Extra Strong), and AC Snowbird, which is the leading cultivar of the new Canada Western Hard White class. AC Superb and AC Snowbird were grown in two locations. All cultivars have red seed coats with the exception of AC Vista and AC Snowbird, which have white seed coats. Cultivar samples were grown in western Canada in the 2001 crop year.

Bran-enriched fractions were obtained by incremental pearling (Satake, Hiroshima, Japan). The pearling consisted of consecutive passages of wheat and pearled wheat. Wheat was initially pearled to remove 5% of the original grain weight, resulting in a first fraction. The remaining kernel was then pearled to remove a second fraction of 5%. The pearlings were continued until the first, second, third, fourth, and fifth fractions (designated 5, 10, 15, 20, and 25%, respectively) plus a residue comprising 75% of the kernel were collected. Wheat samples were also roller-milled using a tandem Buhler mill as previously described by Martin and Dexter (1991). A total of 15 fractions were produced from each genotype designated as break flours (B1–B4, S1), reduction flours (M1–M6, Q1), shorts, bran, and bran flour. Bran flour was produced by passing the bran through a bran finisher and rebolting (183 μm). The mill was set up to give a relatively high extraction rate of 80%. The mill flow and some technological properties of these flour streams have been described (Martin and Dexter 1991). Four of the roller-milling fractions (M1, bran flour, bran, and shorts) were analyzed for comparison with the pearled wheat fractions. Wheat fractions were ground to pass through a 0.5-mm screen.

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Total phenolic content was determined using the Folin-Ciocalteau method (Singleton and Rossi 1965) as modified by Gao et al (2002). Samples (200 mg) were extracted with acidified methanol (HCl/methanol/water, 1:80:10, v/v) (4 mL) at room temperature for 2 hr on a wrist-action shaker (Burrel, Pittsburgh, PA, USA). The mixture was centrifuged at 3,000 rpm for 10 min on a table centrifuge (GLC-1, Sorval, Newton, CT, USA). The supernatant was used for the determination of total phenolics. An extract (0.2 mL) was added to 1.5 mL of freshly diluted 10-fold Folin-Ciocalteau reagent (BDH, Toronto, ON, Canada). The mixture was allowed to equilibrate for 5 min and was then mixed with 1.5 mL of sodium carbonate solution (60 g/L). After incubation at room temperature for 90 min, the absorbance of the mixture was measured at 725 nm. Acidified methanol was used as the blank. Ferulic acid (Sigma, St. Louis, MO, USA) was used as the standard. The results were expressed as ferulic acid equivalents. All tests were duplicated.

Antioxidant activity was measured using a modified version of the Brand-Williams et al (1995) method that involves the use of the free radical 2,2-diphenyl-l-picrylhydrazyl (DPPH), where antioxidants are allowed to react with the stable radical in a methanol solution. The discoloration of the DPPH radicals was followed by monitoring the decrease in its absorbance at a characteristic wavelength during the reaction. In its radical form, DPPH absorbs at 515 nm, but upon reduction by an antioxidant species, the absorption disappears. Ground wheat fractions (0.1g) were extracted with methanol (1 mL) for 2 hr as described above. The extract (0.1 mL) was reacted with 3.9 mL of a 6×10^{-5} mol/L of DPPH solution (2.4 mg of DPPH in 100 mL of methanol). Absorbance (A) at 515 nm was determined at 0 and 30 min. Methanol was the blank. Antioxidant activity was calculated as % of discoloration = $(1 - [A \text{ of sample}_{t=30}/A \text{ of control}_{t=0}]) \times 100$. All tests were duplicated.

Statistical Analysis

The general linear model procedure of the Statistical Analysis System (v. 8.2, SAS Institute, Cary, NC, USA) was used for data analysis. Means were compared at the 5% significance level using Fisher's least significant difference (LSD). Based on LSD, three significant figures were reported for total phenolic content.

RESULTS

Total Phenolic Content of Pearled Wheat Fractions

Total phenolic content in pearled wheat fractions differed significantly among samples with values of 1,300–5,300 mg/kg (Table I). The decrease in phenolic content was progressive as successive pearlings progressed through the aleurone layer and into the inner parts of the kernel. Results gave further confirmation of the location of most phenol compounds in the outer layers of the grain (Fulcher et al 1972). Because the first and second pearlings had the highest levels of phenolics, they were designated as phenolic-enriched fractions. The combined yield of the first two pearling fractions (10%) corresponds closely to the amount typically removed when wheat is debranned before milling (Dexter and Wood 1996).

The phenolic content decreased markedly after the second pearling for most samples because subsequent pearling of the wheat resulted in increased endosperm content. The relationship between phenolic content and % of pearling followed a quadratic model with $R^2 > 0.94$ for all samples. The 5, 10, 15, and 20% pearling fractions had phenolic contents (>3,000 mg/kg) exceeding levels previously reported on millstreams obtained by roller milling (Gao et al 2002).

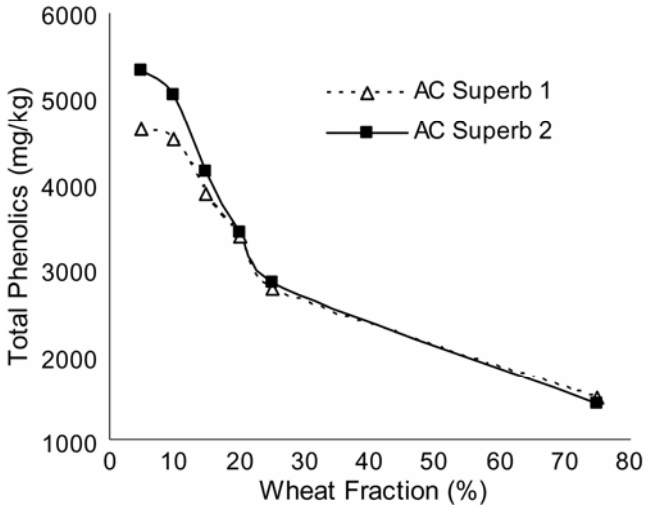

Fig. 1. Effect of environment on total phenolic content of pearled wheat fractions of AC Superb.

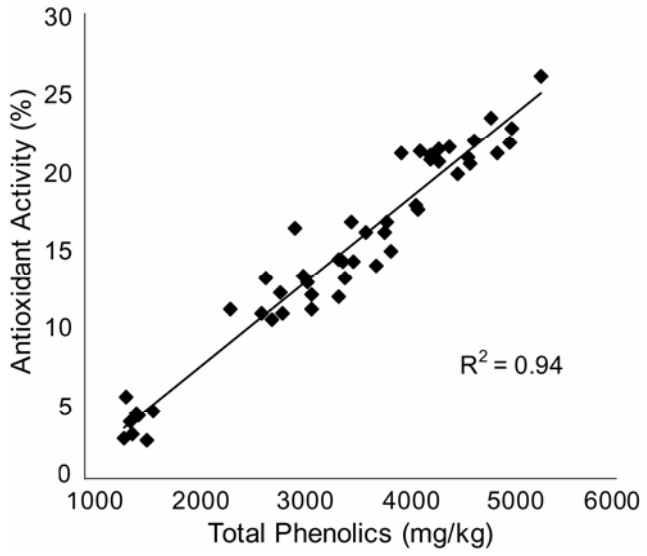

Fig. 2. Correlation between total phenolics and antioxidant activity in pearled wheat fractions ($n = 48$).

TABLE I
Total Phenolics (mg/kg) in Pearled Wheat Fractions Assayed Using the Folin-Ciocalteau Method[a,b]

Fraction	AC Corinne	AC Crystal	AC Barrie	AC Superb 1	AC Superb 2	AC Snowbird 1	AC Snowbird 2	AC Vista	LSD
5%	4,270aDE	4,350aDE	4,840aB	4,640aBC	5,300aA	4,270bE	4,160bDE	4,430bCD	263
10%	4,340aC	3,980bD	5,020aA	4,510aBC	5,020bA	4,900aA	4,620aB	4,680aB	184
15%	3,820bB	2,980cD	3,850bB	3,880bAB	4,140cA	4,130bA	3,640cBC	3,510cD	270
20%	3,450cB	2,690dD	3,740bA	3,380cB	3,430dB	3,380cB	3,520cB	3,060dC	211
25%	2,870dBC	2,350eD	3,140cA	2,770dC	2,840eBC	3,100dAB	3,130dA–C	2,660eC	270
Residue	1,570eAB	1,470fBC	1,620dA	1,490eA–C	1,420fC	1,430eBC	1,380eC	1,350fC	142
LSD	161	232	286	224	259	270	288	192	

[a] Values within the same column with different lowercase letters are significantly different at $P < 0.05$.
[b] Values within the same row with different uppercase letters are significantly different at $P < 0.05$.

Overall, there was no significant difference in total phenolics between red and white wheats for the limited number of samples used in the study. It is worth noting that the analytical method used does not distinguish among the classes of phenolics found in wheat. In studies conducted using sorghum grain, total phenolic content did not correlate with grain or starch color (Beta et al 2001). Assays targeting specific phenolics may yield results that link grain color and certain compounds. For all three white wheat samples (one AC Vista and two AC Snowbird), the 10% fraction had significantly higher phenolic levels than the 5% pearled fraction (Table I). This was not the case for red wheat samples, where similar or higher levels (AC Superb 2) were encountered for the 5% pearled fraction compared to the 10% pearled fraction.

Significant differences in total phenolic content of the 5, 10, and 15% factions were found for AC Superb grown in different locations (Fig. 1). AC Snowbird showed less variation with growing environment. Growing environment is known to affect the levels of other compounds in wheat, including starch and protein. The relative influences of the genotype and environment on phenolic content and antioxidant levels in cereal grains are essentially unknown. As pearling increased to >20%, differences due to growing location were insignificant because phenolics were markedly reduced in the remaining tissues of the kernel.

Antioxidant Activity of Pearled Wheat Fractions

Significant variations in antioxidant activity of pearled wheat fractions were observed (Table II). Antioxidant activity was concentrated in the 5 and 10% fractions as observed with phenolics. Phenolic acids include ferulic acid, vanillic acid, *p*-coumaric acid, and caffeic acid, which are the major antioxidants present in wheat (Onyeneho an Hettiarachchy 1992). Only about half the antioxidant activity of the first or second pearlings remained in the 25% fraction. The residues, representing 75% of the initial sample weight, had much reduced antioxidant activity. Total phenolic content and antioxidant activity of pearled wheat fractions were highly correlated ($R^2 = 0.94$) (Fig. 2), providing strong evidence that the predominant source of antioxidant activity derives from phenolic compounds in wheat. A significant correlation was also found between total phenolics and antioxidant activity of other plant products (Veliogu et al 1998; Zielinski and Kozlowska 2000; Gao et al 2002).

As for phenolic content, there was no significant difference in antioxidant activity of pearled fractions between red and white wheat samples. Residue (75%) of red wheats (except AC Corinne) had similar levels of antioxidant activity. AC Superb gave fractions that differed significantly in antioxidant activity when it was grown in two different environments. There was essentially no variation in antioxidant activity between AC Snowbird grown in different environments.

Phenolics in Roller-Milled Wheat Fractions

The roller-milled wheat fractions were selected to represent three fractions most concentrated in phenolics (shorts, bran, and bran flour) and one (M1) least concentrated in phenolics. M1 represents the purest endosperm fraction that can be obtained through roller milling. The roller mill yields of the selected mill feed and flour streams (corrected to a 14% moisture basis) were shorts 2.1–3.0%, bran 17.3–19.0%, bran flour 1.1–1.5%, and M1 28.1–32.5%. Phenolic content of roller-milled wheats decreased in the order shorts > bran > bran flour > M1 (Table III). This trend likely corresponds to correlated variation in ferulic acid, which is known to be associated with pentosans in the wheat aleurone layer (Fulcher et al 1972) and tends to concentrate in middling flour streams as degree of refinement decreases (Symons and Dexter 1993). Other researchers also reported higher levels of phenolics in bran and shorts than in flour (Abrol and Uprety 1971). The M1 fraction, which represents a highly refined endosperm portion of the kernel, had approximately half the total phenolic content of the 75% residue from wheat pearling. During pearling, inner layers of the crease are left intact, whereas the kernel is essentially ripped open by corrugated break rolls in roller milling. Hence, the former still had significant levels of phenolics contained within the crease. In general, pearled fractions (5 and 10%) had similar or higher levels of phenolics compared with the bran and shorts obtained from roller milling.

CONCLUSIONS

Phenolics were concentrated in pearled fractions representing ≤20% of the outer layers of wheat. Total phenolics and antioxidant activity were highly correlated. Hence, in addition to the well-documented advantages of debranning wheat before milling on product yield and quality (Dexter and Wood 1996), pearling (debranning) wheat before roller milling is an effective technique to obtain wheat bran fractions enriched in phenolic antioxidants. There was no significant difference in total phenolics or antioxi-

TABLE II
Antioxidant Activity (%) in Pearled Wheat Fractions Assayed Using the DPPH Method[a,b]

Fraction	AC Corrine	AC Crystal	AC Barrie	AC Superb 1	AC Superb 2	AC Snowbird 1	AC Snowbird 2	AC Vista	LSD
5%	20.7aC	21.3aC	23.2aB	26.0aC	20.4aA	20.9aC	21.1aC	21.5aC	1.42
10%	20.5aBC	21.0aB	21.7bB	22.6aC	19.7bA	21.1aB	20.8aBC	21.8aB	0.97
15%	15.9bC	16.2bC	16.6cBC	17.5bD	14.7cAB	17.7bA	16.0bC	16.6bBC	0.95
20%	13.0cB	13.0cB	13.8dAB	14.1cC	11.8dA	14.2cA	14.1cA	13.1cB	0.97
25%	10.7dCD	11.0dC	10.9eCD	12.0dD	10.3eB	12.7cA	11.9dB	10.7dCD	0.70
Residue	2.5eC	4.3eB	4.4fB	3.8eB	4.2fB	2.9dC	5.3eA	2.7eC	0.68
LSD	0.87	0.76	0.89	0.69	1.29	1.59	0.85	0.82	

[a] Values within the same column with different lowercase letters are significantly different at $P < 0.05$.
[b] Values within the same row with different uppercase letters are significantly different at $P < 0.05$.

TABLE III
Total Phenolics (mg/kg) in Roller-Milled Wheat Fractions Assayed Using the Folin-Ciocalteau Method[a,b]

Fraction	AC Corrine	AC Barrie	AC Superb	AC Snowbird 1	LSD
Shorts	4,200aB	5,050aA	4,230aB	4,290aB	84
Bran	3,780bB	4,460bA	4,510aA	3,520bB	352
Bran flour	2,870cA	2,510cB	2,590bB	2,680cAB	259
M1	922dA	890dAB	781cBC	741dC	129
LSD	230	167	319	177	

[a] Values within the same column with different lowercase letters are significantly different at $P < 0.05$.
[b] Values within the same row with different uppercase letters are significantly different at $P < 0.05$.

dants between red and white wheat samples used in the studies. However, there was an indication of an influence of growing environment. Further studies are underway to establish the relative effects of genotype and environment on the phenolic content and antioxidant activity.

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LITERATURE CITED

Abrol, Y. P., and Uprety, D. C. 1971. Phenolic constituents in wheat grains. Current Sci. 40:414-415.

Andreasen, M. F., Christensen, L. P., Meyer, A. S., and Hansen, A. 2000. Content of phenolic acids and ferulic acid dehydrodimers in 17 rye (*Secale cereale* L.) varieties. J. Agric. Food Chem. 48:2837-2842.

Beta, T., Rooney, L. W., Marovatsanga, L. T., and Taylor, J. R. N. 1999. Phenolic compounds and kernel characteristics of Zimbabwean sorghums. J. Sci. Food Agric. 79:1003-1010.

Beta, T., Corke, H., Rooney, L. W., and Taylor, J. R. N. 2001. Starch properties as affected by sorghum grain chemistry. J. Sci. Food Agric. 81:245-251.

Brand-Williams, W., Cuvelier, M. E., and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. Lebensm. Wiss. Technol. 28:25-30.

Dexter, J. E., and Wood, P. J. 1996. Recent applications of debranning of wheat before milling. Trends Food Sci. Technol. 35-41.

Faulds, C. B., deVires, R. P., Kroon, P. A., Visser, J., and Williamson, G. 1997. Influence of ferulic acid on the production of feruloyl esterases by *Aspergillus niger*. FEMS Microbiol. Lett. 157:239-244.

Fulcher, R. G., O'Brien, T. P., and Lee, J. W. 1972. Studies on the aleurone layer. I. Conventional and fluorescence microscopy of the cell wall with emphasis on phenol-carbohydrate complexes in wheat. Aust. J. Biol. Sci. 25:23-34.

Gao, L., Wang, S., Oomah, B. D., and Mazza, G. 2002. Wheat quality: Antioxidant activity of wheat millstreams. Pages 219-233 in: Wheat Quality Elucidation. P. Ng and C. W. Wrigley, eds. AACC International: St. Paul. MN.

Hatcher, D. W., and Kruger, J. E. 1997. Simple phenolic acids in flour prepared from Canadian wheat: Relationship to ash content, color, and polyphenol oxidase activity. Cereal Chem. 74:337-343.

Jende-Strid, B. 1985. Phenolic acids in grains of wild-type barley and proanthocyanidin-free mutants. Carlsberg Res. Commun. 50:1-14.

Martin, D. G., and Dexter, J. E. 1991. A tandem Bühler laboratory mill: Its development and versatility. Assoc. Oper. Millers Bull.: 5855-5864.

McCallum, J. A., and Walker, J. R. L. 1990. *O*-diphenol oxidase activity, phenolic content and colour of New Zealand wheats, flours and milling streams. J. Cereal Sci. 12:83-96.

Nishizawa, C., Ohta, T., Egashira, Y., and Sanada, H. 1998. Ferulic acid contents in typical cereals. J. Jpn. Soc. Food Sci. Technol. 45:499-503.

Onyeneho, S. N., and Hettiarachchy, N. S. 1992. Antioxidant activity of durum wheat bran. J. Agric. Food Chem. 40:1496-1500.

Pussayanawin, V., and Wetzel, D. L. 1987. High-performance liquid chromatography determination of ferulic acid in wheat milling fractions as a measure of bran contamination. J. Chromatogr. 391:243-255.

Renger, A., and Steinhart, H. 2000. Ferulic acid dehydrodimers as structural elements in cereal dietary fibre. Eur. Food Res. Technol. 211:422-428.

Singleton, V. L., and Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungistic acid reagents. Am. J. Enol. Vitic. 16:144-158.

Symons, S., and Dexter, J. E. 1993. Relationship of flour aleurone fluorescence to flour refinement for some Canadian hard common wheat classes. Cereal Chem. 70:90-95.

Veliogu, Y. S., Mazza, G., Gao, L., and Oomah, B. D. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J. Agric. Food Chem. 46:4113-4117.

Zielinski, H., and Kozlowska, H. 2000. Antioxidant activity and total phenolics in selected cereal grains and their morphological fractions. J. Agric. Food Chem. 48:2008-2016.

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